UNIVERSITY OF MANITOBA

BACTERIAL NUMBERS AND SUBSTRATE UTILIZATION AS RELATED TO PHYTOPLANKTON ACTIVITY IN WEST BLUE LAKE, MANITOBA

by

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ABSTRACT

Bacterial numbers in West Blue Lake, Manitoba, as determined by plate counting, showed fluctuations of a 14 day or longer duration which were apparently related to changes in chlorophyll a. Seasonal cycles were measured for both bacterial populations and chlorophyll a concentrations. These were found to be similar for some of the depths sampled, particularly the epilimnial depths. Chlorophyll a values in the epilimnion showed spring and mid-summer maxima, with some indication of an impending early fall peak. Bacterial plate counts from Cytophaga medium (Anderson and Ordal 1961) were out of phase relative to chlorophyll a, that is, their peaks, particularly the epilimnial midsummer peaks, succeeded chlorophyll a peaks. The most significant correlation as determined by multiple regression was the negative correlation of temperature with chlorophyll a. Times of maximum temperature change were usually coincidental with maximum carotenoid:chlorophyll a ratio changes.

Although heterotrophic potential was being measured by the direct inoculation of both Cytophaga agar (Anderson and Ordal 1961) and succinate (basal salts solution) agar, comparative measurements were made on a variety of organic acids, glutamate and glucose by means of replica plating from Cytophaga medium. The proportion of Cytophaga agar counts able to use the various media varied with respect to time and substrate. But the mean proportion of each substrate was specific resulting in a relative sequential order as follows; glutamate (highest proportion), glucose, succinate, malate, fumarate, pyruvate, acetate, citrate, tartarate, glycolate and lactate. As substrates in West Blue Lake the latter two are probably insignificant. The proportions varied

from 0% to 50% and higher. The calculated absolute numbers for the replica plating media correlated very highly with Cytophaga agar plate counts.

INTRODUCTION

For many years comparisons have been made between direct microscopic examination and agar plate counts as means of enumerating bacteria. Jannasch and Jones (1959) concluded that the former is a more valid method of estimating the total number of cells. But plate count methods were chosen to estimate bacterial numbers and activity based on the premise that they provide a truer representation of the biochemically active and potentially active heterotrophic planktonic cells.

During the present study investigations were conducted to determine the most suitable media for plate counting and for estimating fluctuations in planktonic bacterial populations in West Blue Lake, Manitoba during 1972. The relationship between the planktonic bacterial population size and phytoplankton activity (chlorophyll and possible soluble organic excretion) was studied.

Although chlorophyll a as a measure of biomass is the topic of much controversy (Parsons and Strickland 1968) it must measure important parameters of the phytoplankton population, considering its importance to algal cell physiology and photosynthesis. That is, chlorophyll a must to some extent estimate algal activity, species composition and possibly biomass.

The carotenoid:chlorophyll a ratio was related to bacterial number fluctuations both quantitatively and qualitatively. This ratio (Yentsch and Vacarro 1958, Manny 1969) and a similar ratio D_{430}/D_{665} (Margalef 1965) is believed to be an indicator of physiological conditions in the phytoplankton community and/or of species composition and its changes.

As such it could be related to the availability of a bacterial

assimilable substrate via primary production and excretion or autolysis or to the release of antibiotic substance.

Both graphical and computer multiple regression analyses and comparisons were carried out between bacterial plate counts, chlorophyll a, the carotenoid: chlorophyll a ratio and temperature.

In lieu of the familiar ^{14}C heterotrophic uptake experiments (Robinson etal 1973), the technique of replica plating (Lederberg etal 1952) was applied to the problem of determining the potential of the bacterial population to utilize single organic acid substrates.

LITERATURE REVIEW

1. Plate counts

The various techniques used in acquiring plate counts and the validity of the counts obtained have been the subject of much debate. Do plate counts underestimate viable numbers due to the inability of the inoculating technique in dispersing bacterial clumps? Are the media used relatively suitable for enumeration? Do they sustain all or a large proportion of the in situ viable cells? Do plate counts grossly underestimate in relation to direct counts and which is ecologically more significant? Collins (1963) and Overbeck (1964) briefly discuss the latter question.

Although spread plates are more convenient than pour plates, the standard recommended 0.1 ml. inoculum restricts effective counting to populations of 300 or more cells/ml (Clark 1971). He found that drying the plates to an optimum loss of 3 g. increased the possible inoculum size and rate of absorption. The recovery of viable colony numbers was independent of the time delay prior to spreading (10 minutes) and of the method of spreading. That is, spreading can be equally effectively continued until all of the inoculum is absorbed or residual wetness can be left to absorb on its own. In the latter case the spreading rod itself retained less than 4% of the colony forming organisms, after spreading for a mere 15 seconds.

Buck etal (1960 and 1961) concluded that the spread plate is superior to the poured plate and that the spreader retained an insignificant proportion of the inoculated bacteria. Jones (1970) found that the proportion of residual bacteria on the spreader amounted to less than 5% ($\bar{x}$ = 2%).

Rodina (1963) mentions bacterial cell clumping. Seki (1971) concluded that the plate count method is not valid as an indicator of microbial biomass due to the large variation in the total number of cells and of heterotrophic bacteria alone per microbial clump.

Surface active agents such as "Tween 80" (Buck etal 1961) have been tested as a means of dispersing bacterial clumps. At 20 ppm it was found to be inhibitory and at 10 ppm there were count increases, but not uniform. Jones and Jannasch (1959) comparing 4 surface active agents showed that "Tween 80" used in conjunction with pour plates produced maximal numbers (range of 1 - 10 ppm). The proportion of clumped bacteria was generally quite low and varied from depth to depth, but usually less than 13% of the total clumped cells were removed. Christensen and Cook (1972) using "Tween 20" found the most effective final concentration in the inoculating solution to be 0.1 ul/l. Counts of cytophagas were 2- 3 1/2 times higher with "Tween 20" than without. Between 1 and 1000 ul/l "Tween 20" had an inhibitory effect.

The use of a plate count procedure raises the question as to how many bacterial colonies per plate constitute a biologically and statistically valid number? Plate counts of milk, water and food (Murphy and Tucker 1970) have a precision of $\pm$ 25%. The standard range of acceptable counts has frequently been stated as being 30 to 300/plate. Counts of less than 30 may be biased due to a high proportion of contaminants, while counts above 300/plate are too difficult to count. An excess of colonies per plate will create competitive forces due to overcrowding (Cooper etal 1968) and may literally eliminate the growth of some. Collins and Willoughby (1962) restricted the range to the limits of 40 to 60 colonies per plate. Buck etal (1960) disputed the assertion that

spread plates routinely contain excessive aerial contaminants.

Jannasch and Jones (1959), using pour plates, found that direct counting produces counts as much as 3 approaching 4 orders of magnitude higher. Atsuki and Hanya (1972) state that plate counts underestimate by 1 to 2 orders of magnitude. According to Jannasch and Jones (1959), the discrepancy is the result of aggregated bacteria, selective effects of the media and the presence of inactive cells.

The length of the incubation period is also an area of discussion. Strzelczyk etal (1968) found that plate counts were still increasing after 10 days and that 14 days was optimal. Jones (1970) observed counts increasing until 23 days of incubation, but along with Strzelczyk etal (1968) found that increased incubation temperature resulted in decreased maximum counts, but a shorter interval preceeding the maximum.

It is preferable to use a medium which will consistently produce maximal numbers. Work done on the effect of altering both inorganic and organic constituents of media (Jones 1970) indicated that the inorganic alterations are more significant. The medium yielding maximum counts in this instance was CPS (casein-peptone-starch medium) as used by Collins and Willoughby (1962). Fonden (1968) testing YPA (yeast extract peptone agar) found that varying the concentrations of the yeast extract or peptone produced insignificant number changes. Comparisons of various media suggested that nutrient poor media are more efficient with respect to sustaining the growth of the bacteria of oligotrophic waters. Strzelczyk etal (1967) tested 7 media and observed that the iron peptone agar yielded the highest counts.

Cytophaga agar (Anderson and Ordal 1961) supports the growth of both eubacteria and myxobacteria. Christensen and Cook (1972) tested

Cytophaga agar and although not judged to be the best medium is a good medium for the enumeration of cytophagas. The addition of SLS (sodium lauryl sulfate) to some of the media inhibited the growth of both cytophagas and non-cytophagas. It was concluded that as a differential counting tool for cytophagas this medium (SLS) was unsuccessful .

2. Aquatic bacteria

The bacterial community consists of different groups of organisms of which plate counts may enumerate only a small proportion. Sorokin (1961) states that different groups of bacteria are distinguishable on the basis of external biosynthetic carbon dioxide requirements. Heterotrophs utilize external carbon dioxide as 3% to 5% of their total carbon metabolism. They are responsible for dark metabolism and assimilation of carbon dioxide in oligotrophic lakes and near the surface of eutrophic and mesotrophic lakes. Bacteria intermediate between true heterotrophs and true chemoautotrophs utilize between 30% and 90% external carbon dioxide in biosynthesis. They oxidize low molecular weight compounds such as methane, methanol, and formic acid. The true chemoautotrophs utilize the oxidation energy of reduced inorganics such as; H_2, H_2S, NH_4, and Fe^{++}. Photosynthetic bacteria may operate in the deeper layers of the euphotic zone if anaerobic conditions exist.

Of the groups mentioned above plate count methods may select for only a small proportion of the community presumed to exist in the lake. Drabkova (1965) enumerating heterotrophic bacteria by means of MPA (meat extract peptone agar) found that they constituted only 0.05% to 0.3% of the total number of bacteria estimated by direct counts.

Collins (1960) classifies lake bacteria into 3 categories:

1. They may be of allochthonous origin and may expire or adapt and

survive the new environment.

2. They may be of autochthonous origin existing in the lake's dilute nutrient solution, that is, planktonic forms using low concentrations of soluble organics as substrates.

3. They may exist on solid surfaces such as soil particles, mud detritus or plankton.

They may be active or inactive, reactivated by the stimulus of a medium. These statements provide the basis for possible discrepancy between actual occurrences in situ and that revealed by plate counts.

The active bacteria (Straskrabova - Prokesova 1966) are defined as being "bacteria capable of reproduction within 3 hours and the bacteria divided a short time before sampling." This number was found to vary from 10.1% to 68.5% of the total direct counts. Seki (1971) found that bacterial biomass as estimated by direct counts agrees well with biomass estimates by the glucose uptake method. He is insinuating that the viable heterotrophic micro-organisms either compose a major proportion of the total number and/or that the heterotrophs remain a constant fraction of the total. Sorokin (1968) in a lake situation estimates the number of saprophytes as being 15% of the total number of bacteria, the remaining 85% being autotrophs.

3. Pigment determinations and phytoplankton biomass

The procedure of pigment determination is subject to many sources of error. The presence of myxophyceae and their attendant phycobilin pigments maybe co-extracted and will interfere with all but the determination of chlorophyll a (Strickland and Parsons 1968). The use of 90% acetone may not completely extract all cellular pigments. During extraction some species may retain 50% of their pigment (Strickland and

Parsons 1968). In a marine situation most values are acceptable except for some chlorophyta and some benthic species. In freshwater situations Strickland and Parsons (1968) recommend checking the efficiency of extraction, if it were possible.

The presence of chlorophyll degradation products will also interfere in the precise determination of pigments (Strickland and Parsons 1968). Corrections can be made for these pheo-pigments (Moss 1967, Strickland and Parsons 1968, Lorenzen 1967, and Banse and Anderson 1967), whose presence is particularly significant in freshwater (Moss 1967).

Long and Cooke (1971) in a comparison of GF-A, GF-C and HA membrane filters found that glass fibre filters were superior with respect to filtering time, time of extraction and the quantity of pigment extracted. Their results show that quite possibly membrane filters may retain more detritus and extracellular products. After treatment by maceration the membrane filters apparently tended to retain some unbroken and partially fragmented cells with undissolved pigment, while glass fibre filters were void of pigmented cellular remains.

Due to the equation of chlorophyll a and biomass a discussion of the validity of this is necessary.

Chlorophyll a can vary in algae by a factor of 5 (Strickland 1960) dependent solely on the prehistory of nutrition and illumination. Chlorophyll a:carbon ratios vary from species to species by at least an order of magnitude. The "F" values vary from 10 to values larger than 100, but these values are quoted from various authors using different methods. There appears to be no correlation between biomass of algae and their constituents, particularly chlorophyll a (Pavoni 1969). Mullin etal (1966) showed no linear correlation between chlorophyll a

and carbon. Evans and McGill (1970) found a good positive correlation between chlorophyll a and carbon.

Thomas (1972) observed that as the growth rate was increasing the carbon:chlorophyll a ratio decreased and began to level off at about 50% of the maximum growth rate. McAllister etal (1961) and Antia etal (1963) noticed that as the culture aged, the carbon:chlorophyll a ratio increased.

Steele and Baird (1962) found a carbon:chlorophyll a ratio in winter of 213:1 and a maximum in autumn of 47:1. In July and August in Loch Nevis the carbon:chlorophyll a ratio was 74:1. During the spring peak in the northern North Sea the carbon:chlorophyll a ratio was 23:1. This variation may be due to species composition differences.

Chlorophyll to biomass conversions may vary with depth (Steele 1964) as a result of algal cells low in the euphotic zone having a higher proportion of chlorophyll a. Lorenzen (1968) states that the carbon:chlorophyll a ratio decreases with increasing %Io. The ratio ranges from 39 to 64, with the final mean value being 40.5.

In a comparison of various methods of determining microbial biomass (Holm-Hansen 1969), it was found that there was good agreement of direct examination of biomass, the ATP method and chlorophyll a determinations converted to carbon for 0 through 100 meters.

Strickland (1960) suggests that carotenoids are a better biomass estimator, because they have a more constant relationship to organic matter than does chlorophyll a.

4. Bacterial seasonal dynamics

Although numbers of inactive and/or active bacteria need not correlate with generation time (Straskrabova-Prokesova 1966), the latter is

shortest in spring and fall. Beliatskaia (1958) using total bacterial counts reports summer and winter minima in June and July and January respectively. In all 3 lakes examined the spring increase took place in May. The autumn maximum varied, beginning in August or September and ending in November. Kozhova and Kazantseva (1961) noted that in one year maximal bacterial numbers in Lake Baikal occurred in August and September and the next year the latter half of July and August. The summer maximum being larger than the spring maximum.

Drabkova (1965) mentions that the longest generation times in Red Lake occurred in June, August and February at which times bacterial numbers decreased. The maximum number of bacteria occurred from April to June. The heterotrophs measured using MPA (meat extract peptone agar) relative to the total number of bacteria increased faster in summer and slower from October to March. The heterotrophic number maximum occurred in April and from October to November. Potaenko (1968) demonstrated that bacterial growth was maximal in spring and autumn and minimal in summer and winter. Although the pattern remained the same the actual bacterial numbers as determined by direct counts varied from year to year. Aerobic heterotrophic bacteria (Overbeck 1967) have been reported as reaching a maximum in summer coincident with the phytoplankton peak.

The patterns of bacterial populations are generally very variable both chronologically and geographically. Overbeck and Babenzien (1964) observed that in a small artificial water body, bacterial numbers obtained by direct counts were at a maximum in April, May and in June and September and October. A pronounced summer minimum was a common feature noted by a number of authors. Other authors mentioned by Overbeck etal

(1964) did not detect a significant annual cycle, while others noted a maximum in summer and a minimum in winter.

5. Reasons for bacterial fluctuations

There is evidence for harmonic fluctuations of algae (Steven and Glombitza 1972), and possibly bacterial cycles are due to some direct or indirect influence of the algal fluctuations. These may be long term cycles and/or, as proposed by Saunders and Storch (1971), the total reaction sequence of light, phytoplankton, extracellular soluble organic matter and bacteria may be a linearly coupled fluctuating control system cycling on a daily basis.

It has been demonstrated by Potaenko and Mikheeva (1969) that bacteria and algae may possess 3 possible relationships:

1. Maximum bacterial growth accompanied by minimum algal growth or the inverse.
2. The growth of the 2 may be parallel.
3. Both of the above may occur in one lake.

Bacterial periodicity demonstrates various seasonal patterns as reviewed earlier. Jones (1971) presented a brief review of factors influencing the fluctuations of the bacterial community. Algal periodicity has been well reviewed by Lund (1964 and 1965).

That a relationship of algae and bacteria exists can be shown circumstantially by with depth studies. Fonden (1969) noted that chlorophyll a decreased with depth as did bacterial plate counts on YPA (yeast extract peptone agar). Overbeck (1967) reached similar conclusions and more specifically stated that bacteria depend on substrates released by autolysis and release and/or excretion of soluble organics. Substrates, in turn, exert their influence by increasing growth and

normally the associated reproduction rates of the bacterial community. The number of bacteria present at any one time is a direct function of prior reproduction rates (Straskrabova-Prokesova 1966). Of importance here are active bacteria which were found to vary from 10.1% to 68.5% of the total direct counts. The availability of substrate appears to be more important than temperature with respect to changes of generation time. Drabkova (1965) using direct counts obtained similar results. In February reproduction rates decreased due to a lack of available substrate, while in summer the phytoplankton had a bactericidal effect. In April heterotrophs grown on MPA (meat extract peptone agar) increased at the expense of allochthonous microflora, while at other times increases occurred in response to algal die-offs. Beliatskaia (1958) has also shown that numbers of bacteria are directly related to the amount of organic matter in the water or the trophic state of the system.

The allochthonous microflora (Drabkova (1965) and/or other organic matter may enter as a result of rainfall. The microflora dies quickly resulting in potential substrate material. Collins (1960) and Collins and Willoughby (1962) observed similar effects of rainfall at least down to the thermocline.

In a brief review Potaenko and Mikheeva (1969) mention how algae can influence bacterial communities by altering the environment. Activities include:

1. Competition for soluble organics.
2. Antibacterial effects.
3. Alkalization of water to pH 9-10 by algae, assimilating carbon dioxide in the free and bicarbonate forms.
4. An increase in the redox potential that results from saturation of

the medium with the oxygen released during photosynthesis.

Kozhova and Kazantseva (1961) and Morris and Foster (1971) explain summer bacterial numbers by stating that although algal biomass is low, production is high, resulting in synthesis of particulates, and excretion will act as a limit on the rate of biomass synthesis together with respiration rates. The former authors also suggest that higher summer temperatures may enhance bacterial numbers. Consequently numbers are lower below the thermocline.

Overbeck and Babenzien (1964) determined the correlation coefficient +0.56 between bacterial direct counts and algal direct counts sampled weekly. Other years the correlation was not significant. Generally maxima of both algae and bacteria were found to occur in spring and autumn. It appeared at times that bacterial numbers were increasing as phytoplankton numbers were decreasing. Physical parameters such as substrate levels, solar radiation, precipitation, wind and pH appeared to be of secondary importance in influencing bacterial numbers. Schegg (1968) using direct counts showed that bacterial numbers and primary production are directly correlated, while algal biomass is indirectly correlated to bacterial numbers. He concedes that primary production is partially diverted to excreted products that are used as substrates by bacteria.

In a computer analysis of Esthwaite Water (Jones 1971) bacterial numbers enumerated on CPS agar (casein peptone starch agar) were positively correlated with temperature, dissolved oxygen, pH and negatively with depth. The depth and pH relationships of Windermere were similar, but here bacterial counts also correlated with particulate carbon and nitrogen. A multiple correlation of Esthwaite Water revealed good

agreement between bacterial numbers and a combination of pH and temperature with the chlorophyll a concentration. At constant levels of chlorophyll a, bacteria correlated with temperature and pH, but a relationship between bacteria and chlorophyll a alone could not be demonstrated. Jones (1971) summarizes by stating that in a nutrient rich lake bacteria are controlled by temperature, pH and dissolved oxygen, while in a nutrient poor lake particulate matter, pH and rainfall are the determining factors.

Goldman etal (1968) conducted a computer multiple regression analysis of 28 variables. Unfortunately pigment determinations were not made of the phytoplankton. Phytoplankton biomass determined from microscopic volume measurements, showed that the Bacillariophyceae were almost perfectly correlated with the total phytoplankton and were the major component. The diatoms were negatively correlated with temperature from 0 - 20 meters. From June through August bacterial plate counts were positively correlated to the total phytoplankton biomass and with total zooplankton. Direct counts of bacteria and total phytoplankton were negatively correlated. The smaller the total phytoplankton biomass, the higher the rate of production of new biomass measured by ^{14}C uptake. High production of extracellular products of photosynthesis tends to occur as the phytoplankton population declines. Temperature was not significantly correlated to either bacterial plate counts or direct counts.

Although it has been shown that maybe there is a relationship between bacteria and phytoplankton, particularly by the release of soluble organics by the latter, it would still be desirable to know at what times excretion or autolysis and release are occurring. De-

creases of chlorophyll a may be related to particulate matter decreases and a resultant increase in soluble organics. But this need not explain all soluble organic release. Measurements of the physiological state of algae might indicate when excretion and autolysis are occurring and affecting the bacterial community. Species changes may also influence the release of soluble organics.

Both the physiological state and the species composition can to some extent be quantified jointly by the pigment ratio as used by Margalef (1961 and 1965) of D_{430}/D_{665}. The analysis of the ratio in a heterogeneous community is difficult since both species composition and the physiological state influence it simultaneously. Other authors have attached similar significance to the carotenoid:chlorophyll a ratio. For instance the Bacillariophyceae tend to have the lowest ratio and are probably the major component of the total algal community (Parsons etal 1961). Dehadrai and Bhargava (1972) observed that a low chlorophyll a:carotenoid ratio signified the presence of a chlorotic unhealthy phytoplankton population.

Investigations relating nitrogen deficiency and subsequent recovery to algal physiology and the carotenoid:chlorophyll a ratio have been done by Yentsch and Vacarro (1958), McAllister etal (1961) and Manny (1969). Loss of nitrogen due to deficiency resulted in a more rapid loss of chlorophyll a than carotenoids, while recovery from nitrogen deficiency was accompanied by a more rapid gain of chlorophyll a.

Quoting Margalef (1965), "A high inorganic carbon uptake per unit biomass is always associated with a low biotic diversity or with a low pigment ratio D_{430}/D_{665}." The correlation coefficient between production and chlorophyll a concentration was found to be +0.964. As the

rate of production increases, green pigments increase more rapidly than yellows and the pigment ratio may drop.

Of particular significance to bacterial fluctuations in West Blue Lake are facts concerning phytoplankton composition and periodicity in this lake. The most common species (Cliff M.S. 1972) are Anabaena spiroides, Aphanizomenon flos-aquae, Asterionella formosa, Botryococcus braunii, Dinobryon sertularia, and Cyclotella bodanica. The succession as determined during 1970 and 1971 consists of maxima of D. sertularia, A. formosa and C. bodanica in May and June. The latter is significant throughout the summer. In early July the phytoplankton drop to a minimum. During the middle of July and August Aphanizomenon flos-aquae and Anabaena spiroides become dominant.

6. Heterotrophy and replica plating

In any study of aquatic bacteria it should be specified exactly what group of organisms is presumably being distinguished and studied. According to Collins (1960) there are 3 categories of lake bacteria, "those washed in from outside sources, of which a large proportion will ultimately perish but some will find conditions suitable for growth; indigenous types capable of existence in a dilute nutrient solution as represented by lake water, and able to use for growth low concentrations of available organic matter; and those dependent on a solid surface for their proliferation and therefore found in connection with soil particles, mud detritus and plankton."

The bacterial group that replica plating in this thesis deals with are those included in the second group above, and those which according to Sorokin (1969) have been transferred in and which are able on artificial media being used, that is the first group mentioned above (Collins

1960). Sorokin (1969) maintains this selection will create misleading results as to the true nature of dynamics in the lake. The bacteria being replicated are those classified by Sorokin (1961) as being true heterotrophs or intermediates between true heterotrophs and true chemotrophs.

Replica plating according to the method of Lederberg and Lederberg (1952) can be used to determine cultural characteristics of various bacterial types. It is questionable whether the results obtained by this method will accurately represent the lake bacteria or whether the colonies produced have adapted or mutated on the original plate being replicated until able to use specific substrates replicated onto. Are the populations in the lake currently capable of utilizing the same substrates? If so, are these substrates actually present in the lake?

Glucose was chosen as a replica plating substrate due to its ubiquitous use in bacteriological media. The 9 organic acids used were chosen for various reasons including availability and mention in the literature, for example, glycolic acid. The amino acid glutamate was chosen as a result of Gocke's work (1970) showing a decline of glutamate concentrations excreted by Scenedesmus quadricauda when bacteria were present. In the axenic cultures glutamate was one of the dominant excretory products.

When bacteria (Gocke 1970) are added to a culture, the dissolved free amino acids are reduced to a level 1.8% of the original. In axenic cultures glutamate and aspartate dominated, but in non-axenic cultures as in a lake situation, serine and glycine are the dominant free amino acids. The addition of certain amino acids to a Scenedesmus quadricauda culture showed the specificity of this alga as compared to bacteria with

respect to the uptake of substrates. The algae almost completely removed threonine, serine, glycine and alanine from solution, while aspartate and glutamate concentrations introduced, remained virtually unchanged.

Halvorson (1972) studied the utilization of single amino acids by soil bacteria. The natural community was best able to utilize threonine, aspartic acid and glutamic acid. The substrates histidine, tyrosine, alanine, aspartic acid and proline permitted the growth of the best developed colonies. Generally glutamic acid produced only moderately well developed colonies. In natural situations (Halvorson 1972) amino acids do not normally accumulate and can serve as sole sources of both carbon and nitrogen in the complete absence of growth factors.

Berland etal (1970) in a study of bacteria isolated from marine algal cultures showed that of the substrate classes used, particularly in the absence of growth substances, amino and organic acids were utilized by bacteria more frequently than sugar and sugar derivatives.

Storch (M.S. 1971) found excellent agreement between the rate of release of dissolved organic carbon by phytoplankton and the assimilation of the same by bacteria. Quantities released by the algae were generally less than 10% of the total net carbon fixation. The release rates were related to the growth rate and species composition of the phytoplankton. A large proportion of this is assimilated by bacteria.

Table 1: Turnover time in hours for nine organic acids at a depth of 5 meters during July and August, 1971 at Station 5, West Blue Lake (Henzel M.S. 1972).

Relative position	Organic acid	Turnover time
1	Malate	0
2	Fumarate	75
3	Citrate	250
4	Pyruvate	295
5	Lactate	300
6	Acetate	375
7	Succinate	460
8	Formate	700
9	Glycolate	950

Henzel (M.S. 1972) investigated the ^{14}C uptake of 9 organic acids in West Blue Lake, Manitoba. Ranking the acids according to turnover times (Table 1) should approximate the efficiency of the heterotrophic population in utilizing each acid. Of most importance would be malic and fumaric acid, while glycolate would be least important. Other evidence was found that supported the concept of only non-algal uptake of the acids. Depth effects on uptake varied for the various acids, but it appears as though specific bacterial populations exist and take up specific substrates.

Research at West Blue Lake in 1971 (Robinson, Henzel and Gillespie 1973) demonstrated remarkable correlation ($r = 0.98$) between agar plate counts inoculated directly using the substrates pyruvate, fumarate, malate, acetate, glycolate and citrate and their respective ^{14}C acid maximum velocities of uptake. Two other acids succinate and lactate did not conform to the above pattern. It appears that lactate may be primarily an algal substrate. Succinate supports the largest proportion of bacteria and is unusually widely accepted (Robinson etal 1973).

SUMMARY OF LITERATURE REVIEW

Although care must be taken in the interpretation of agar plate counts particularly when they are low, they are the best means of determining numbers of viable active heterotrophic bacteria. In West Blue Lake and probably most others, direct microscopic counts are essentially useless for investigations of bacterial activity. Direct counts (Gillespie, pers com) are invalidated by the presence of dead and inactive cells and other confusing detrital particles. Since the lake is clear particles are not numerous, although detrital particles do outnumber algal particles. Bacterial cells probably exist as single planktonic cells.

Cytophaga (Anderson and Ordal 1961) plate counts (Gillespie, pers com) consistently produce maximum numbers of colonies. The colony forming cells are heterotrophs, probably the major proportion of the bacterial community of West Blue Lake, Manitoba.

Anaerobes such as methane oxidizers or autotrophs are not likely to exist because of a relatively high oxygen content. Since West Blue Lake is dimictic oxygen is being introduced and being oligotrophic, the oxygen is never depleted to anaerobiosis.

A significant proportion of these heterotrophs, however, may be non-indigenous. The cells may be washed in by rainfall, underground inflows or the two temporary inflows of West Blue Lake.

Previously the pattern of bacterial fluctuation in West Blue Lake (Gillespie, pers com) was apparently following a 14 day cycle. Experiments were intended to determine how consistently this occurred and if so, why? In other situations almost every conceivable pattern, usually of a more long range nature has been found. The pattern seen most often

consists of spring and fall maxima, with summer and often winter minima. The results of this investigation will permit a comparison of West Blue Lake with these works.

The phytoplankton of West Blue Lake (Cliff M.S. 1972) and those mentioned in the literature are also known to undergo cyclic fluctuations. These can be related to bacterial fluctuations and have been found to be both direct and indirect.

In West Blue Lake both biomass and species composition form a reasonably consistent pattern from year to year (Cliff M.S. 1972). This may be the result of temperature changes associated with dimixis and may be associated with consistent changes in the carotenoid:chlorophyll a ratio and the carbon:chlorophyll a ratio changes of the phytoplankton. These changes may be the basic determining factors of the magnitude of the bacterial community and its activity.

This relationship may occur by way of algal release of organic substrates, assimilable by the heterotrophic bacterial community. From the literature and former ^{14}C uptake experiments at West Blue Lake, a number of substrates were chosen to use in determining bacterial utilization, by means of directly inoculated plate counts onto succinate containing agar and replicated onto a variety of substrates by replication from Cytophaga (Anderson and Ordal 1961) plates. This is comparable with results in the literature, usually using in situ uptake of ^{14}C labelled substrates, and particularly with past results from West Blue Lake. The latter is a comparison of preference orders of substrates with the maximum velocities of uptake of ^{14}C labelled compounds.

METHODS

Description of sampling area

West Blue Lake, Manitoba, is centrally located in the Duck Mountain Provincial Park, approximately 300 miles northwest of the city of Winnipeg, Manitoba. It is of glacial pleistocene origin, being a variant kettle-type multi-basin channel lake. Of its three basins, the northern-most is 20 meters deep (location of Station 5), the central attains a maximum depth of 31 meters (site of Station 2) and the southern basin is 18 meters deep. The lake is 4.8 kilometers long and 0.52 kilometers wide at the widest point. The mean depth is 11.3 meters and its area is 160 hectares. The shoreline development is 2.87. The lake is essentially self contained except for 2 minor inflows which flow during spring or periods of heavy rainfall. These are located in the southern most basin and in the central basin, directly north of Station 2.

Enumeration of bacterial numbers in West Blue Lake

The apparatus used to collect samples for bacterial plate counts consisted of a one liter PVC van Dorn sampler suspended from a trolling rod. It was stored and transported between the laboratory and sampling sites within a plastic bag inside a steel cylinder. The entire sampling apparatus was sterilized prior to use by means of ethylene oxide ampules.

The samples were collected between 0845 hrs and 0920 hrs from Station 2 at weekly intervals. Sampling was commenced at 30 meters and then sequentially towards the surface. A minimum of contact was maintained between the apparatus and sources of contamination during sampling. The depths sampled were 0,1,3,5,7,12,17,25 and 30 meters. The

approximately 100 ml. samples were carefully piped off through a 110 u filter into 125 ml. screw cap autoclaved sample bottles. The 9 samples so obtained were conveyed in an insulated wooden box, ensuring a minimum of contact with direct sunlight.

The media used for the above inoculations were Cytophaga (Anderson and Ordal 1961) agar, tryptic soy agar (TSA), succinate agar with basal salts and sodium lauryl sulfate (SLS) agar. The last is a modification of Cytophaga medium and is similar to the medium of Christensen and Cook (1972).

The formulae of the media are as follows:

1. Cytophaga agar	pH 7.2
Tap water	1000 ml.
"Difco" Bacto-tryptone	2.0 g
"Difco" Bacto-beef extract	0.5 g
"Difco" Bacto-yeast extract	0.5 g
Sodium acetate	0.2 g
"Difco" Bacto-agar	9.0 g
2. Sodium lauryl sulfate agar	pH 7.2
Tap water	1000 ml.
"Difco" Bacto tryptone	2.0 g
"Difco" Bacto-beef extract	0.5 g
"Difco" Bacto-yeast extract	0.5 g
Sodium acetate	0.2 g
Sodium lauryl sulfate	0.5 g
"Difco" Bacto-agar	9.0 g

3. Tryptic soy agar ("Difco"), final pH 7.3 at 25°C.

"Difco" Bacto-tryptone	15 g
"Difco" Bacto-soytone	5 g
Sodium chloride	5 g
"Difco" Bacto-agar	15 g
Tap water	1000 ml.

4. Succinate agar with basal salts agar pH 7.2

Distilled water	1000 ml.
K_2HPO_4	0.7 g
KH_2PO_4	0.3 g
$MgSO_4$	0.2 g
$CaCl_2$	0.04 g
$FeCl_3$	0.01 g
KNO_3	2.0 g
Sodium succinate	5.0 g
"Difco" Bacto-agar	10.0 g

Note: The media used for replica plating were the same as No. 4, but with various substrates replacing sodium succinate.

When dissolved at a pH of 7.2 the media were autoclaved, then cooled gradually in a water bath to approximately 47°C prior to pouring. In a laminar flow chamber each disposable plate received about 17 ml. of medium and plates were usually prepared 2 days prior to inoculation. The samples were treated with 0.1 ml. of 0.1% "Tween 20" (1971 - 0.1 ml. of 1.0% "Tween 20") and placed on a shaker at 300 rpm for 10 minutes. Each sample was then diluted by pipetting 10 mls. into a sterile 90 ml. dilution blank.

The original samples and the 10^1 dilutions (after hand mixing) were

inoculated onto the agar surfaces and spread with the aid of a turntable and a spreading rod immersed in 70% alcohol and flamed before use. Cytophaga agar and TSA were each inoculated with 0.1 ml. of the 10^0 and 10^1 dilutions. Succinate agar and SLS agar were only inoculated with the 10^0dilution. For each dilution at each depth 3 replicate plates were inoculated. Inoculation in the laminar flow chamber took place from 0930 hrs to approximately 1130 hrs.

The inoculated plates were incubated at 20°C for 5 days. Colony counts were obtained using a simple Quebec colony counter. The entire plate was counted.

The individual plate counts were multiplied by the respective dilution to obtain the number of colonies per milliliter. For comparative purposes the mean counts of both dilutions at each depth were used.

A number of evaluative experiments were performed during 1971 for which the methods varied to some extent from the regular inoculations of 1972.

On June 29th and July 1st, 1971 a numerical comparison was made between 8 points at a depth of 7 meters about each of Stations 2 and 5 and consequently between the stations themselves. Four plates of Cytophaga agar were used for each of the 10^0 and 10^1 dilutions for each of the 16 points.

Two experiments were designed to determine the magnitude of bacterial fluctuations when the sampling interval was shortened, in the first case samples were taken every second day and in the second case every 3 hours for approximately 1 day.

The first experiment was initiated July 7th and ended July 23rd, 1971. Sampling was accomplished between 0900 hrs and 1000 hrs. The

media used were Cytophaga agar, tryptic soy agar and sodium lauryl sulfate agar. Five replicate plates of each of the 10^0 and the 10^1 dilutions were used.

The second experiment was carried out twice, that is, between August the 9th and 10th and between August the 23rd and 24th, 1971. The media used were Cytophaga agar, TSA and SLS agar. The former began at 0700 hrs and samples were taken every 3 hours until 0100 hrs of the 10th and a final isolated sample at 1000 hrs. The latter began at 0700 hrs with samples taken every 3 hours until 1000 hrs of the 24th. For the former Cytophaga agar and TSA were inoculated from both the 10^0 and 10^1 dilution, 4 replicates each being used. For SLS 4 replicates were inoculated with 0.1 ml of the 10^0 dilution and 4 with 0.2 ml of the same. In the latter only the 10^0 dilution was used, 8 replicates of Cytophaga agar and TSA and 4 for SLS agar.

REPLICA PLATING

A single Cytophaga agar plate was chosen from each of 5 depths; 0, 3, 7, 17 and 30 meters for replica plating. The plates were subjectively chosen using 2 criteria:

1. The plate should, if possible, be reasonably representative of the population at that depth, including colonial types and relative proportions of types.
2. The plate should be amenable to replica plating, that is, colonies should be quite spatially distinct from each other to prevent smearing during transfer, possible syntrophism and to facilitate easy analysis. Due to this the replica plating plates frequently have less than the mean number of colonies for that depth.

Prior to replication the 5 selected Cytophaga agar plates were

carefully mapped and the colonies numbered together with a brief description.

The replica plating sequence was as follows; the selected Cytophaga agar plate transferred onto succinate, glucose, glutamate, malate, pyruvate, acetate, citrate, fumarate, tartarate, glycolate, lactate and onto another Cytophaga agar plate. The last plate served as a control to determine that all of the colonies were successfully transferred.

The transfers were accomplished (Lederberg and Lederberg 1952) using velvet squares of cloth autoclaved in a large glass beaker covered by aluminum foil. The squares were stretched taut over a wooden block with the aid of a large hose clamp. The entire procedure was carried out in the laminar flow chamber, 6 days after the respective initial direct Cytophaga agar inoculation.

The proportion of the colonies successfully transferred able to utilize a specific substrate was assessed on a subjective basis. Since the plate replicated tends to have fewer than the mean number of colonies, the proportion determined above was multiplied by the mean Cytophaga agar count at that particular depth to obtain the number per milliliter able to utilize a particular substrate.

ALGAL PIGMENTS

The 2 liter samples were obtained using a 3 liter van Dorn sampler. The samples were taken, beginning at 30 meters and progressing towards the surface through the following depths; 0, 1, 3, 5, 7, 12, 17, 25 and 30 meters. The samples were taken from Station 2 between 1500 hrs and 1530 hrs, avoiding direct sunlight on the samples.

In a procedure similar to that of Parson and Strickland (1968), the 2 liter samples were filtered through glass fibre GF-A filters

immediately following sampling. One milliliter of 1.0% magnesium carbonate suspension was added to the last few hundred milliliters of sample being filtered. The filters were placed in ground glass stoppered centrifuge tubes containing 10 mls. of 90% reagent grade pre-filtered acetone. These were stored in a dark refrigerator for 20 hours to allow extraction of the pigments.

After 20 hours the extracts were refiltered through GF-A glass fibre filters and made up to 12 milliliters. The absorbance was determined in a Unicam SP-500 spectrophotometer using the following wavelengths; 750 mu, 665 mu, 645 mu, 630 mu 510 mu and 480 mu. The readings were completed within 2 hours. Until June 25, 1972 the 1 cm. cuvettes were used, after which a change was made to 10 cm. cuvettes.

Spectrophotometric readings were corrected for cell to cell and turbidity blanks (Parsons and Strickland 1968).

Chlorophyll a in ug/l was determined according to the formula of Parsons and Strickland (1968);

$C(\text{chl a}) = 11.6\ E_{665} - 1.31\ E_{645} - 0.14\ E_{630}$ all divided by 2 to correct for the 2 liter samples.

Carotenoids measured in m-SPU/1 (mille-specified plant unit) were determined according to the formula of Richards (Parsons and Strickland 1968);

$C(\text{plant carotenoids}) = 7.6\ (E_{480} - 1.49\ E_{510})$ all divided by 2 to correct for the 2 liter samples used.

RESULTS

a. Variance of plate counts

In order to estimate the validity of plate counts, determinations of means, standard deviations, standard errors and the range were compared for representative sampling dates of 1972 (Tables 2, 3 and 4). High mean counts tended to be associated with low relative standard deviations of frequently less than 25% of the mean. When plate counts were less than 10 the standard deviation approached the mean or exceeded it. Examples of high mean counts were the plate counts from the 12th of May at 3 meters, the 10th of July at 1 meter and of low mean counts were the plate counts from the 5th of June at 5 meters and the 21st of August at 12 meters. Exceptions included the high standard deviations of the 12th of May at 3 and 7 meters and the 31st of July at 3 meters. Two experiments (June 29 and July 1, 1971) demonstrated differences of means for the 10^0 and the 10^1 dilution of samples when inoculated onto Cytophaga agar (Table 5 and 6). They also demonstrated horizontal spatial differences of counts sampled from points near 2 stations. These plate counts provided many examples of large ranges between the counts indicating large standard deviations, which decreased confidence in the results. Another comparison of Stations 2 and 5 (September 9, 1972) provided many good examples of small ranges in the plate counts with the major exceptions as expected, being the counts of 12 and 17 meters at Station 2. The small ranges were the basis for confidence in the mean counts.

Table 2: Means, standard deviations, standard errors and the ranges of Cytophaga agar plate counts in numbers per milliliter for representative sampling dates throughout the sampling season of 1972 at Station 2, West Blue Lake, Manitoba.

	12/5/72				15/5/72			
Depth	Mean	St.Dev.	St.error	Range	Mean	St.Dev.	St.error	Range
0	1612	434	177	1000-2280	582	221	90	300-840
1	837	239	98	600-800	380	222	91	100-600
3	5350	3146	1285	1500-10,000	988	258	115	660-1310
5	1648	610	249	800-2410	812	441	180	400-810
7	2060	1063	434	1290-3900	692	228	93	300-930
12	617	271	111	300-1100	787	418	171	340-1500
17	562	288	117	260-1100	280	120	49	100-400
25	285	152	62	100-540	682	184	82	530-1000
30	395	110	45	300-600	187	79	32	100-320

	22/5/72				29/5/72			
Depth	Mean	St.Dev.	St.error	Range	Mean	St.Dev.	St.error	Range
0	257	104	42	100-400	77	52	21	0-150
1	187	125	51	90-400	88	78	32	0-200
3	78	67	27	0-170	137	130	53	50-400
5	133	90	37	0-260	88	90	37	0-200
7	302	149	61	100-500	150	177	72	0-500
12	198	153	63	90-500	80	67	27	0-200
17	410	348	142	160-1100	98	113	46	0-300
25	118	75	31	0-500	80	29	12	30-100
30	182	169	69	0-500	112	62	25	20-200

Table 3: Means, standard deviations, standard errors and the ranges of Cytophaga agar plate counts in numbers per milliliter for representative sampling dates throughout the sampling season of1972 at Station 2, West Blue Lake, Manitoba.

	5/6/72				10/7/72			
Depth	Mean	St.Dev.	St.error	Range	Mean	St.Dev.	St.error	Range
0	155	131	53	40-400	318	100	41	200-500
1	122	90	37	60-300	1912	154	63	1720-2100
3	25	28	11	0-60	205	106	43	100-400
5	47	76	31	0-200	290	237	97	0-700
7	58	39	16	0-100	2967	699	285	200-3700
12	15	28	11	0-70	157	133	54	0-400
17	193	139	57	0-400	827	98	40	700-940
25	120	70	28	0-190	303	105	43	200-500
30	237	55	22	180-300	270	48	21	200-310

	31/7/72				14/8/72			
Depth	Mean	St.Dev.	St.error	Range	Mean	St.Dev.	St.error	Range
0	183	131	53	100-440	2242	277	113	1840-2600
1	248	104	42	100-400	700	117	48	550-860
3	477	653	267	110-1 800	1747	419	171	1200-2300
5	112	93	38	0-210	393	121	50	200-510
7	322	292	119	200-900	273	122	50	150-500
12	100	64	26	0-200	165	87	36	60-300
17	312	169	69	0-500	218	136	56	0-400
25	307	112	46	200-500	433	184	75	300-800
30	147	82	34	0-210	498	216	88	300-900

Table 4: Means, standard deviations, standard errors and the ranges of Cytophaga agar plate counts in numbers per milliliter for representative sampling dates throughout the sampling season of 1972 at Station 2, West Blue Lake, Manitoba.

	21/8/72				4/9/72			
Depth	Mean	St.Dev.	St.error	Range	Mean	St.Dev.	St.error	Range
0	140	51	21	100-210	245	166	68	0-500
1	262	198	81	0-600	432	54	24	240-500
3	240	179	80	0-500	288	170	69	0-500
5	310	144	64	100-500	755	259	106	460-1100
7	85	76	31	0-200	662	76	31	600-800
12	27	37	15	0-100	323	162	66	200-600
17	177	73	30	100-300	127	74	30	0-200
25	338	203	83	100-700	218	68	28	100-300
30	623	166	68	300-740	240	157	64	0-400

DISCUSSION

a. Variance of plate counts

Very few conclusive recommendations can be made concerning the results mentioned and plating procedure. Care must be taken in the interpretation of all plate counts. Although relatively speaking standard deviations could be quite high, the counts of 1972 were assumed to be reasonably accurate. A basic problem in the study of the bacterial community of West Blue Lake was that mean viable counts were low, so low, that frequently even the inoculation of undiluted samples with only 3 replicates did not produce counts that, considering the variance, appear statistically reliable. That is, many individual plate counts were less than the minimum recommended per plate, 30 colonies. But as mentioned in results, as means increased, generally so did the reliability. And it is precisely these counts which this thesis is primarily interested in, high counts and the reasons for their being high.

Usually plate counts were less than 300 and the biological effects of overcrowding were not likely to have affected accurate determinations of the mean.

Although frequently the means of the 10^1 dilution were higher than the 10^0 dilution, it was not thought to be due to either contamination or to dispersal of clumps after dilution. The occurrence of the latter could have been detected readily. It was assumed that the former was not occurring to any significant extent. The 10^1 dilution had a high variance due to low counts thus causing the variation in mean counts frequently seen between the 2 dilutions as mentioned above. Nevertheless both dilutions were used for calculations.

RESULTS

b. Horizontal homogeneity of bacterial numbers

Two experiments of June 29 and July 1, 1971 (Tables 5 and 6) were designed to determine not only whether 2 distinct sampling stations widely separated, had similar bacterial counts at the same depth, but also whether points about Stations 2 and 5 (that is, simulating being off station while sampling) varied significantly from each other. The results varied depending on the time of the experiment. June 29, the counts were similar for both stations and for the points about each station (Table 5). On July 1st (Table 6) the stations and points about each station were apparently dissimilar.

In the experiment of September 9th, 1972 (Table 7) plate counts were available for all sampling depths for both Stations 2 and 5 as opposed to only 7 meters for the above. The data demonstrated station differences from 0 through 17 meters indicating lack of homogeneity even in the hypolimnion.

DISCUSSION

b. Horizontal homogeneity of bacterial numbers

Considering that at times the 8 sampling points about each station did vary significantly in terms of plate counts, care should be taken in regular sampling, that samples are taken as near the station as possible.

It is doubtful whether it could ever be assumed that statistically any 2 points at one specific depth in the lake were the same. Even if counts were similar it is doubtful whether the community composition would be. If exceptions to this did occur, this would most probably be

during periods of overturn or possibly in the winter, but even the latter is doubtful. Too many physical phenomena are present that may cause significant differences between Stations 2 and 5 such as the inflow just north of Station 2 and the wind induced currents of Station 5 at least in the epilimnial strata.

Data from one or more sampling stations cannot be extrapolated to represent the entire lake.

RESULTS

c. Temporal dynamics

For the experiments conducted on the 9th and 10th of August and on the 23rd and 24th of August, 1971 (Table 8) samples were taken from Station 2 at a depth of 7 meters every 3 hours and inoculated onto Cytophaga agar, tryptic soy agar and sodium lauryl sulfate agar plates. The results were subjected to the "F test" to determine whether changes in bacterial numbers were significant on a short term basis relative to the variance encountered within the individual plate counts for each sampling time. Although the counts of both experiments (Cytophaga agar counts for August 9th and 10th and tryptic soy agar counts for August 23rd and 24th) were significant at least at the 5% level of significance, the F values were close to the rejection region and the results of the 2 experiments were not comparable with respect to any sort of diurnal pattern.

Another experiment conducted from July 7th to July 23rd, 1971 involved sampling every 2 days and inoculating the samples onto Cytophaga agar, tryptic soy agar and sodium lauryl sulfate agar (Table 9). The samples were taken from 7 meters at Station 2. The "F-test" was applied

to test the significance of bacterial number changes relative to the variance within the individual plate counts. The F values for both Cytophaga agar and tryptic soy agar were very significant.

Table 5: June 29, 1971 Cytophaga agar plate counts from points taken approximately equidistant from Station 2 and Station 5 and the nearest shoreline and from points of similar distances in the remaining two compass directions, at 7 meters in West Blue Lake (No/ml - number per milliliter).

STATION 2

Points	10° dilution Plate counts	No./ml.	10' dilution Plate counts	No./ml.	Mean No./ml.
E	7,8,12,11	95	1,1,3,-	166	131
E'	12,16,12,15	137	1,2,2,-	166	152
S	14,0,4,9	67	0,0,1,0	25	46
S'	16,5,25,15	152	0,2,0,0	50	101
W	6,6,7,29	120	0,0,0,1	25	73
W'	4,7,2,-	43	6,-,0,2	266	155
N	14,6,1,1	55	3,5,2,5	375	215
N'	2,3,1,2	20	1,6,1,0	200	110

STATION 5

E	4,3,1,5	33	1,0,0,1	50	41
E'	2,6,8,1	43	0,2,2,-	133	81
S	22,9,12,17	150	1,2,4,2	225	188
S'	20,28,26,15	223	1,8,0,3	300	261
W	32,18,19,13	205	2,4,1,0	175	190
W'	12,7,16,6	103	3,-,2,2	233	159
N	25,12,25,12	185	2,1,0,1	100	143
N'	2,5,3,5	38	0,3,0,0	75	56

Table 6: July 1, 1971 Cytophaga agar plate counts from points taken approximately equidistant from Station 2 and Station 5 and the nearest shoreline and from points of similar distances in the remaining two compass directions at 7 meters in West Blue Lake (- uncountable) (No./ml. - number per milliliter).

STATION 2

	10^{0} dilution		10^{1} dilution		Mean
Points	Plate counts	No./ml.	Plate counts	No./ml.	No./ml.
E	4,1,2,2	23	0,-,0,-	0	12
E'	0,3,3,6	30	0,5,0,0	125	78
S	34,58,54,88	585	12,8,10,7	925	755
S'	15,12,11,12	125	0,4,1,4	225	175
W	6,1,22,6	88	1,1,1,6	225	157
W'	67,97,71,107	855	11,13,22,14	1500	1178
N	--	--	--	--	--
N'	4,14,8,14	95	2,1,1,0	100	98

STATION 5

E	--	--	--	--	--
E'	--	--	48,66,119,110	8575	8575
S	--	--	23,59,49,63	4850	4850
S'	--	--	45,84,66,65	6500	6500
W	--	--	80,48,70,70	6700	6700
W'	--	--	2,35,33,21	2275	2275
N	--	--	7,7,11,13	950	950
N'	--	--	7,21,15,11	1350	1350

Table 7: Comparison of Cytophaga agar plate counts for September 9, 1972 for Stations2 and 5 at West Blue Lake.

Depth	Station 2		Station 5	
	Plate counts[a]	Mean[b]	Mean[b]	Plate counts[a]
0	39,38,47	413	497	42,65,42
1	32,30,39	337	510	53,42,58
3	123,160,147	1430	570	68,--,46
5	35,36,32	343	407	46,37,39
7	32,37,31	300	1007	124,88,90
12	3,8,7	60	1263	152,122,105
17	8,6,3	57	880	80,85,99
25	17,12,--	150		
30	25,30,35	300		

(a) Counts per individual plates (10° dilution).

(b) Calculated numbers per milliliter.

Table 8: Plate counts in numbers per milliliter from samples taken every three hours during two distinct days (August 9-10, 1971 and August 23-24, 1971) at Station 2 from 7 meters, West Blue Lake(TSA - tryptic soy agar, SLS - sodium lauryl sulfate).

	August 9-10			August 23-24	
Time	Cytophaga agar	TSA	SLS	TSA	SLS
7 a.m.	20	15	3	470	3
10 a.m.	31	13	4	141	0
1 p.m.	36	15	4	265	0
4 p.m.	149	109	9	206	0
7 p.m.	50	44	6	290	0
10 p.m.	70	20	8	449	3
1 a.m.	6	29	8	340	0
4 a.m.				413	3
7 a.m.				603	3
10 a.m.	154		31	496	0

Cytophaga agar (August 9-10) with 7 + 56 d.f. F = 6.943
5% level of significance 2.17 - 2.25[a]
1% level of significance 2.95 - 3.12[a]
TSA (August 23 - 24) with 9 + 69 d.f. F = 2.221
5% level of significance 1.96 - 2.04[b]
1% level of significance 2.72 - 2.56[b]

(a) Table values for 60 - 40 degrees of freedom
(b) Table values for 120 - 60 degrees of freedom

Table 9: Plate counts taken every two days during July 1971 (Counts in numbers per milliliter) from 7 meters at Station 2, West Blue Lake.

Date	Cytophaga agar	TSA	SLS
July 7	78	35	24
July 9	200	40	16
July 11	825	60	38
July 13	505	112	48
July 15	158	81	15
July 17	430	227	11
July 19	2565	1449	636
July 21	275	430	27
July 23	194	40	6

Cytophaga agar with 8 + 78 d.f. F = 105.906

5% level of significance 2.10 - 2.02_a

1% level of significance 2.82 - 2.66_a

TSA with 8 + 78 d.f. F = 63.484

5% level of significance 2.10 - 2.02_a

1% level of significance 2.82 - 2.66_a

(a) Table values for 120 - 60 degrees of freedom.

c. Temporal dynamics

On a short term basis (within 24 hours) changes in bacterial numbers were barely significant and considering errors, that can arise from plate count variance or simply having sampled slightly off station, created a lack of confidence in the validity of apparent number changes.

Although changes within 24 hours were not really significant, day to day changes in bacterial numbers were very significant. For the detection of bacterial number cycles sampling every 2 days or preferably even every day would be desirable. The apparent 8 day cycle that occurred during the interval July 7th to July 23rd, 1971 would probably not be detectable by weekly sampling. Weekly sampling may show unreal cycles on account of this.

RESULTS

Cytophaga agar plate count fluctuations and possible contributing factors

The Cytophaga agar plate counts were graphically compared to chlorophyll a, the carotenoid:chlorophyll a ratio and temperature (Figures 1 - 9). The data included here was grouped (Appendix 1) and analyzed by multiple regression omitting days 133 and 248. Depth comparisons are presented of means and standard deviations (Table 10) and of the corresponding simple correlation coefficients. (Table 11).

Generally from 0-25 meters (Figures 1-8), it appeared that the pattern of bacterial numbers, ignoring weekly fluctuations, is quite similar. From the middle of May to sometime early in June, Cytophaga agar plate counts fell rapidly. Following this in an oscillating manner the counts rose, forming a gradual peak extending into early September.

Five meters differed from the other depths (0 - 7 meters) in that it never manifested a peak with numbers exceeding 1000 cells per milliliter. Individual Cytophaga agar count peaks such as the July 10th peaks at 1 and 7 meters tended to distort the common pattern.

Numbers of bacteria, particularly in the surface strata, probably seldom remained static for any significant period of time. The numbers at each depth oscillated widely and frequently apparently quite independently of each other. Although the phenomena had been seen previously (Gillespie pers com), bacterial numbers never established a regular approximately weekly oscillation enduring more than a month.

Unlike bacterial depth profiles, chlorophyll a formed a number of intergrading but distinctive depth profiles throughout the 1971 summer season. The definition of these patterns is primarily decided by the pigment concentrations at 12 and 17 meters.

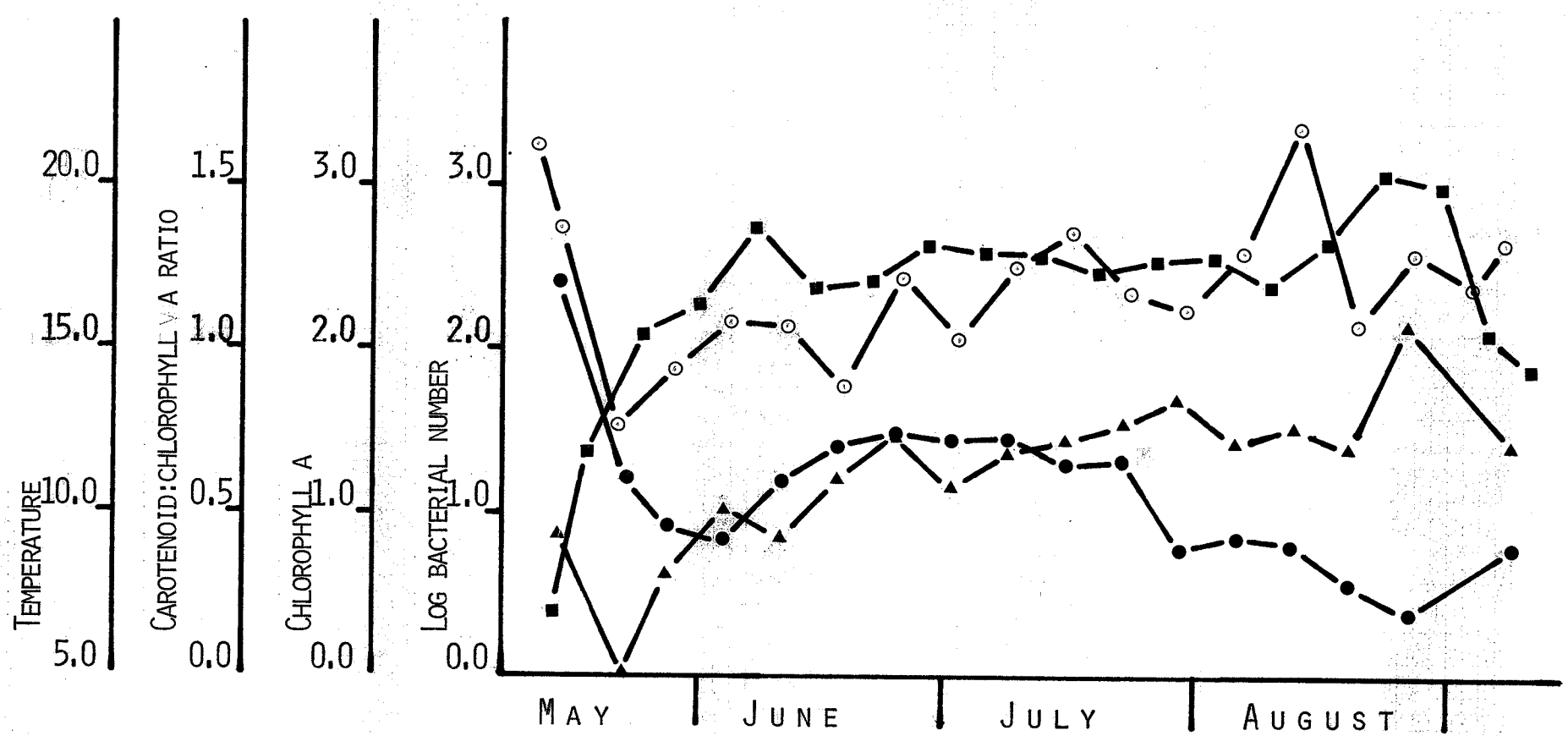

FIG.1: A COMPARISON OF 4 PARAMETERS FOR 0 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

○ — ○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
● — ● CHLOROPHYLL A (UG./L.)
▲ — ▲ CAROTENOID:CHLOROPHYLL A RATIO
■ — ■ TEMPERATURE (°C)

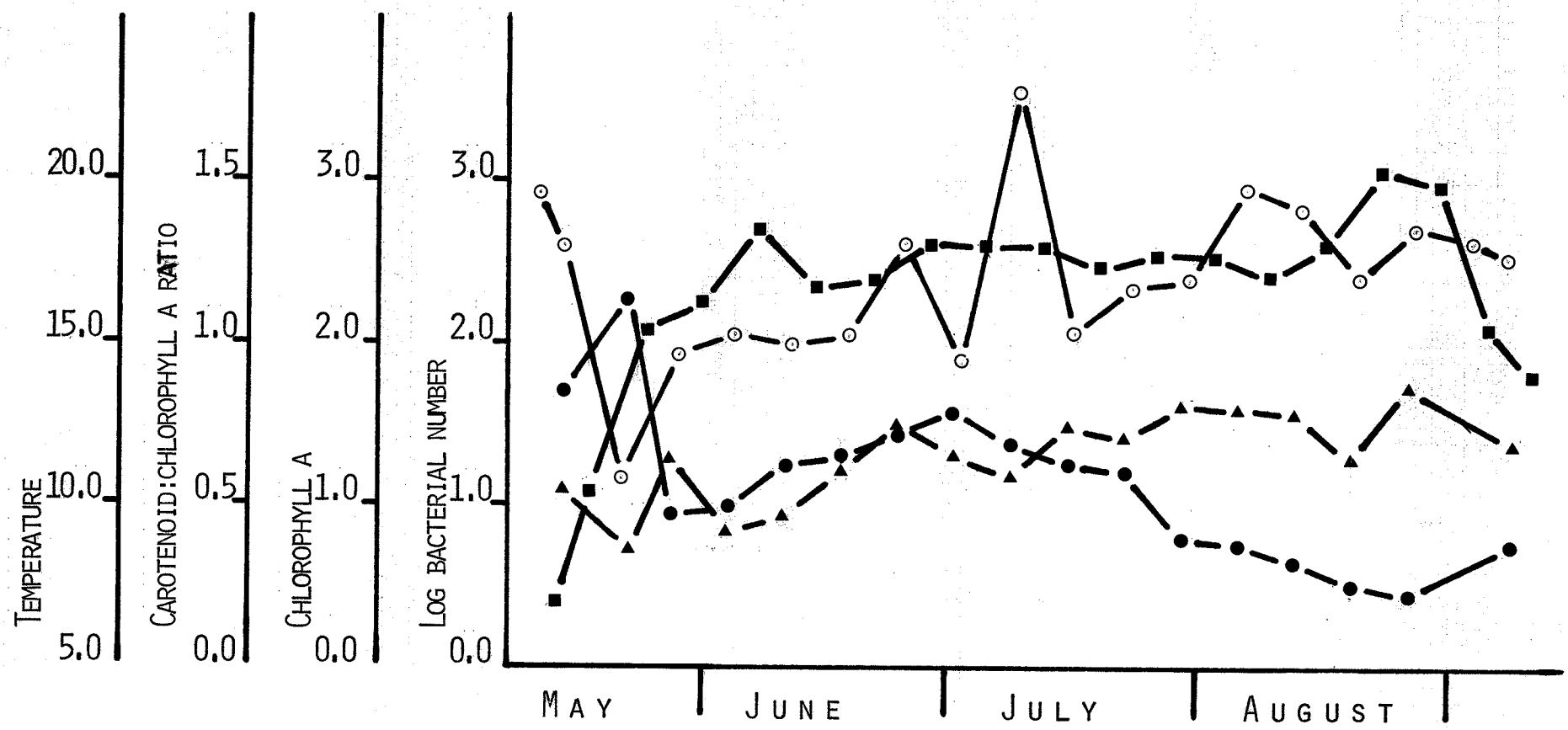

FIG.2: A COMPARISON OF 4 PARAMETERS FOR 1 METER, 1972, IN WEST BLUE LAKE AT STATION 2.

○—○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
●—● CHLOROPHYLL A (UG./L.)
▲—▲ CAROTENOID:CHLOROPHYLL A RATIO
■—■ TEMPERATURE (°C)

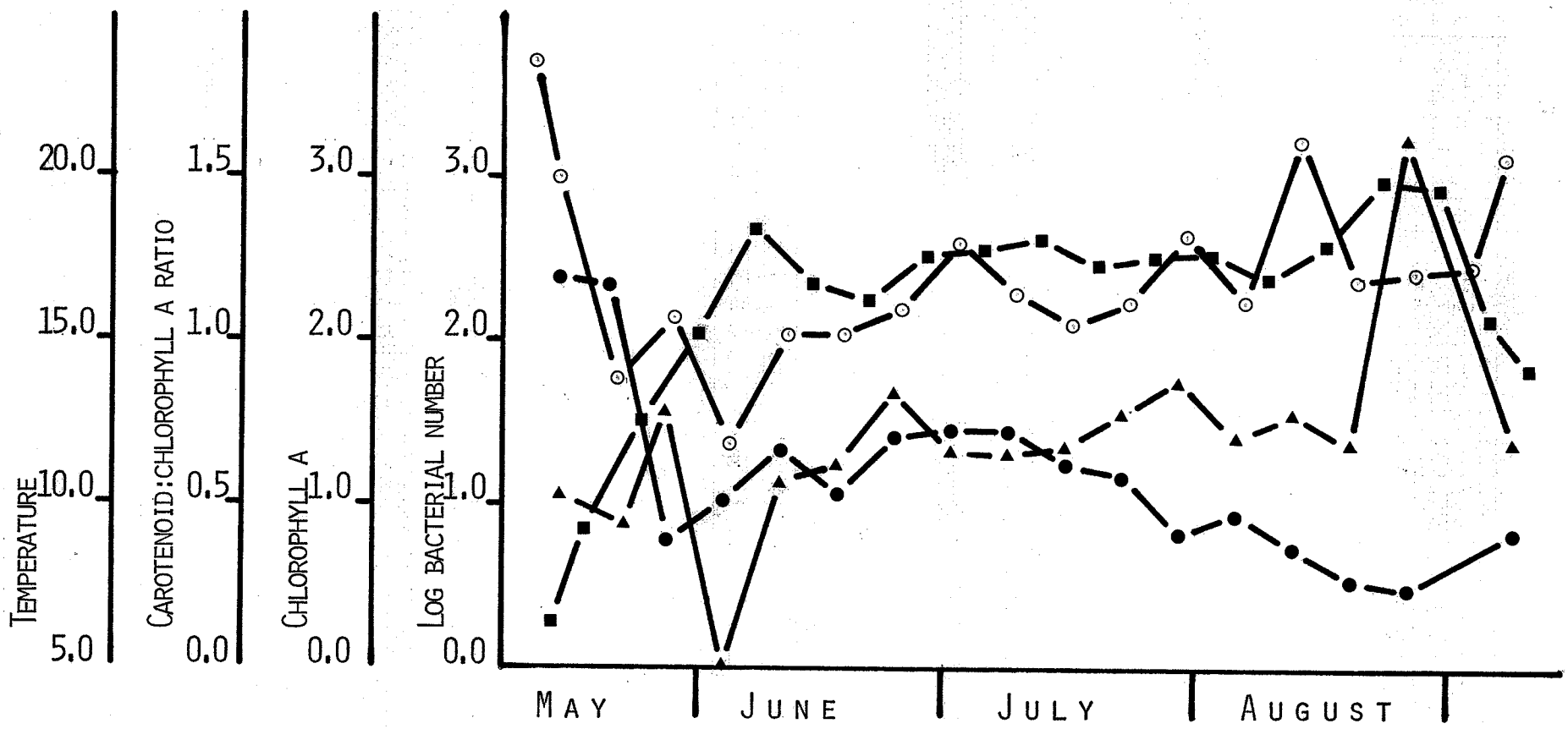

FIG.3: A COMPARISON OF 4 PARAMETERS FOR 3 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

○—○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
●—● CHLOROPHYLL A (UG./L.)
▲—▲ CAROTENOID:CHLOROPHYLL A RATIO
■—■ TEMPERATURE (°C)

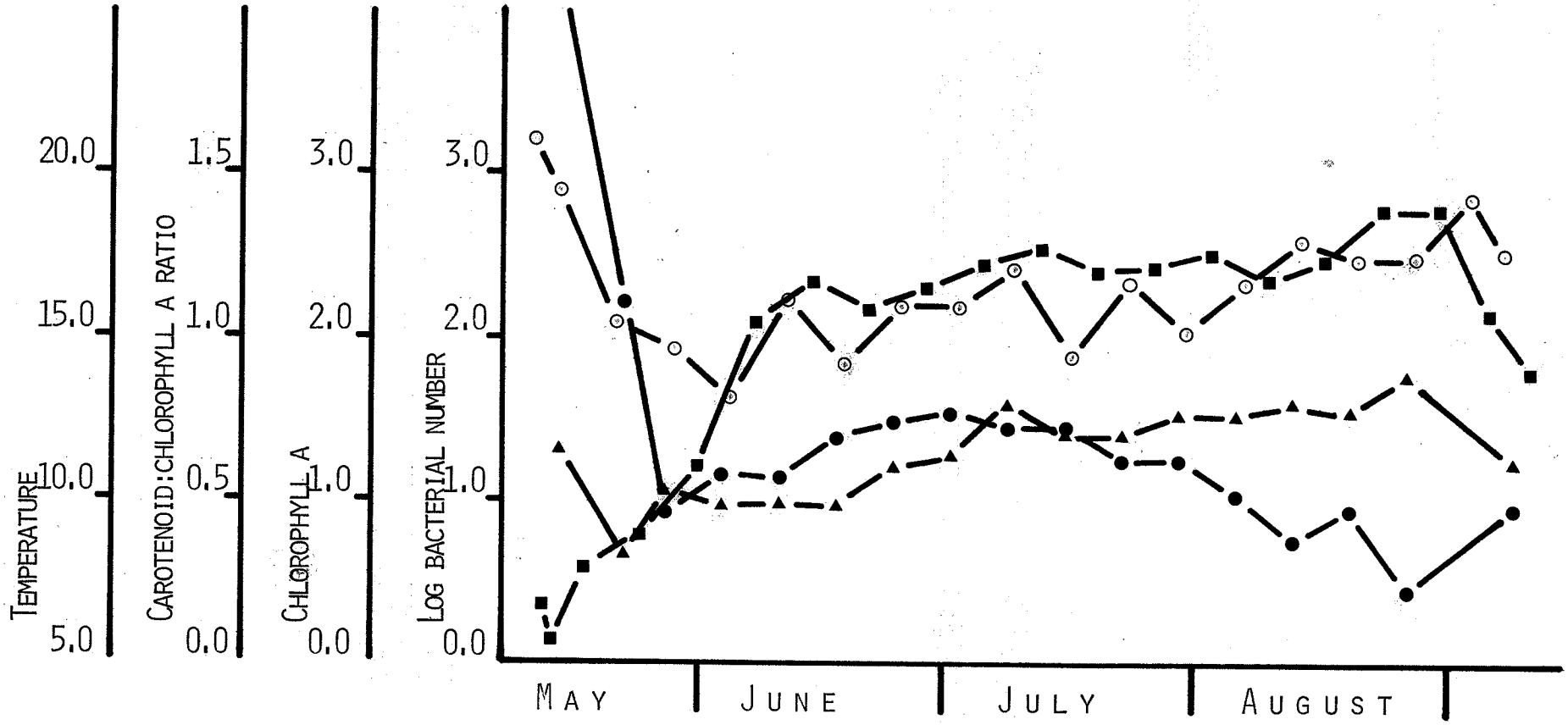

FIG.4: A COMPARISON OF 4 PARAMETERS FOR 5 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

○—○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
●—● CHLOROPHYLL A (UG./L)
▲—▲ CAROTENOID:CHLOROPHYLL A RATIO
■—■ TEMPERATURE (°C)

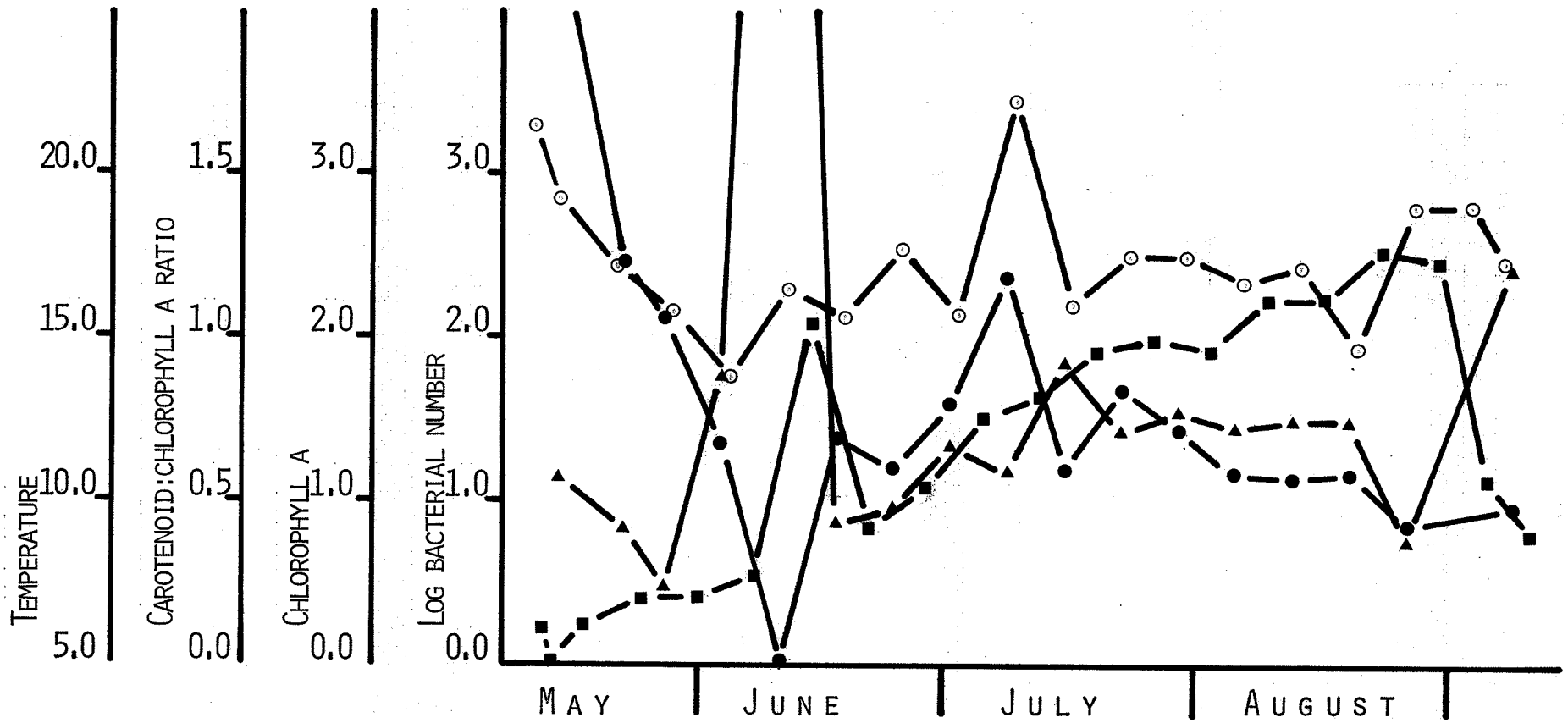

FIG.5: A COMPARISON OF 4 PARAMETERS FOR 7 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

○—○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
●—● CHLOROPHYLL A (UG./L)
▲—▲ CAROTENOID:CHLOROPHYLL A RATIO
■—■ TEMPERATURE (°C)

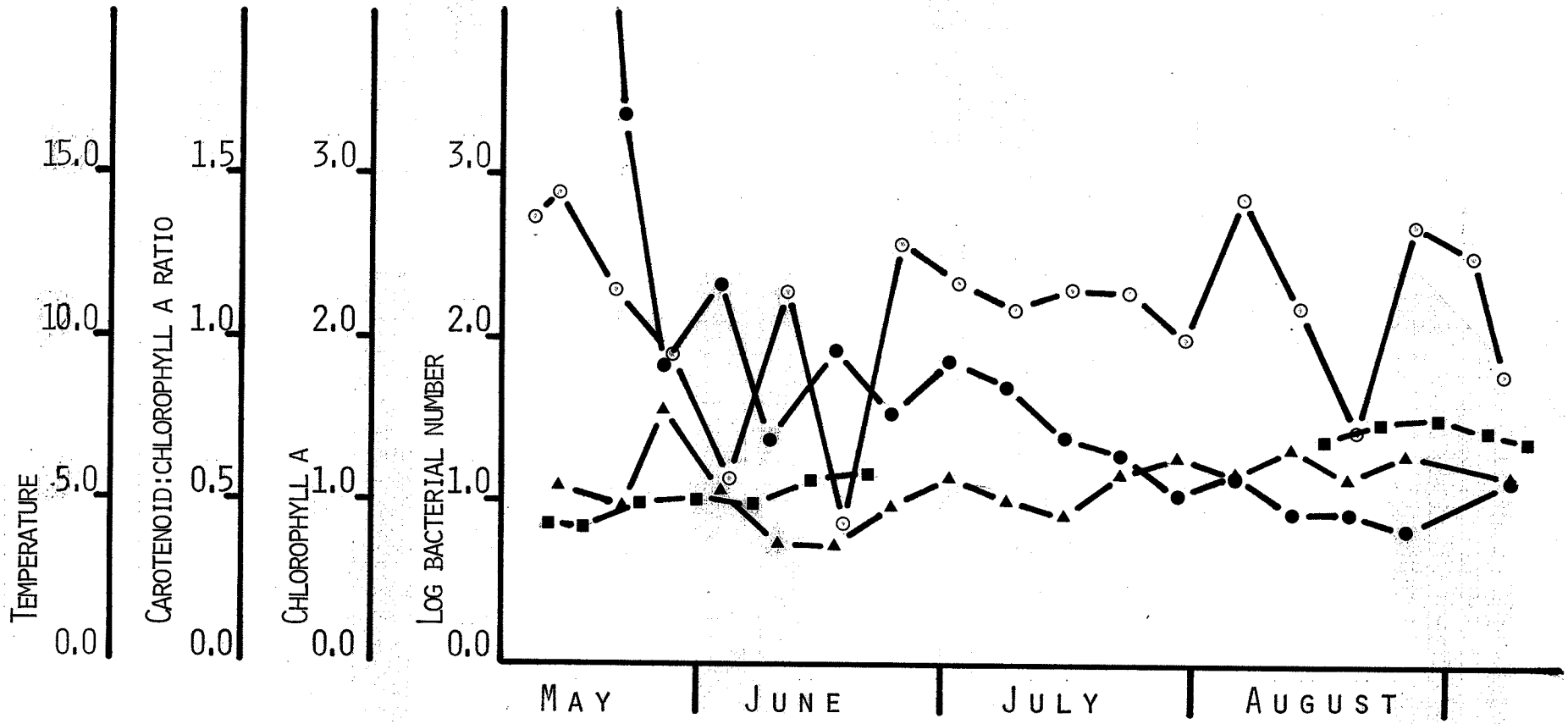

FIG.6: A COMPARISON OF 4 PARAMETERS FOR 12 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

○—○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
●—● CHLOROPHYLL A (UG./L.)
▲—▲ CAROTENOID:CHLOROPHYLL A RATIO
■—■ TEMPERATURE (°C)

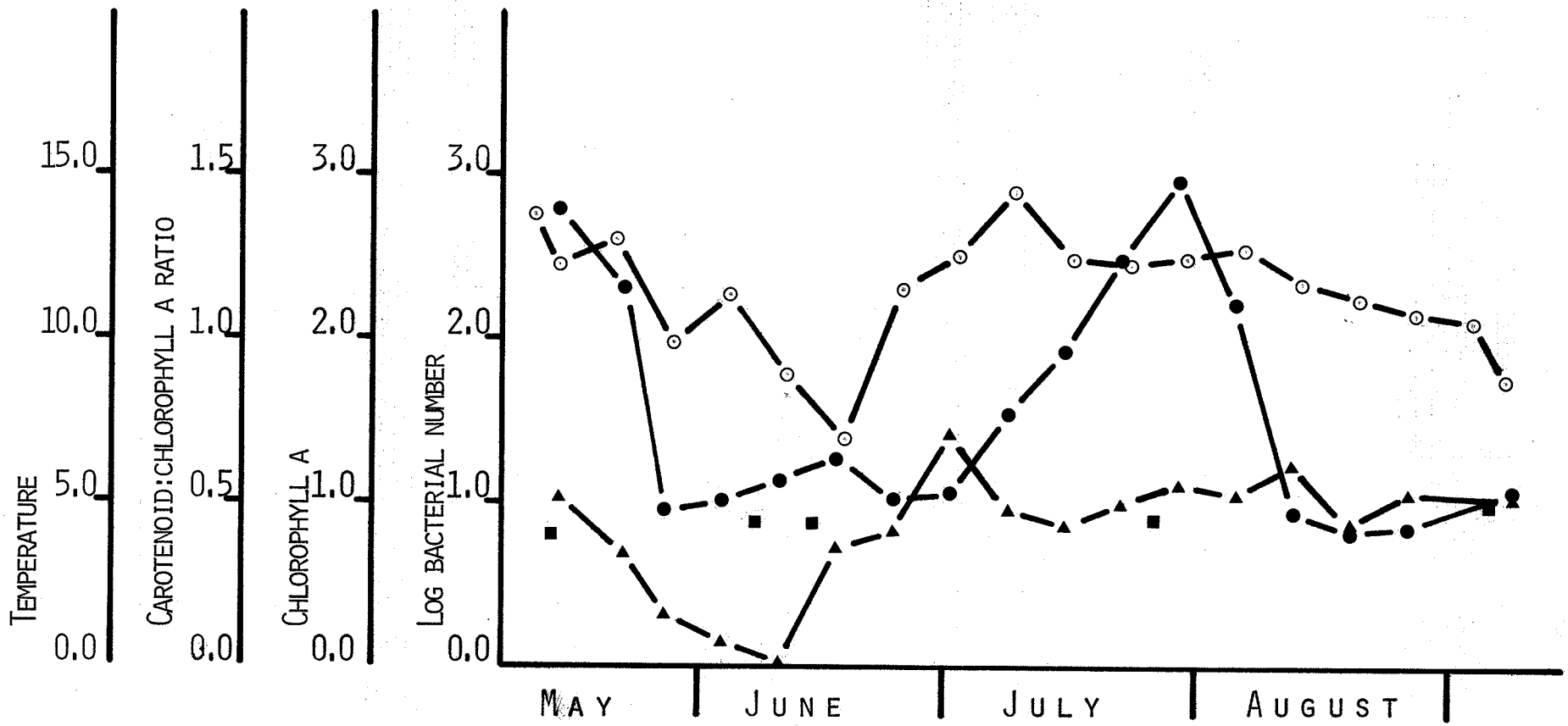

FIG.7: A COMPARISON OF 4 PARAMETERS FOR 17 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

○—○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
●—● CHLOROPHYLL A (UG./L.)
▲—▲ CAROTENOID:CHLOROPHYLL A RATIO
■—■ TEMPERATURE (°C)

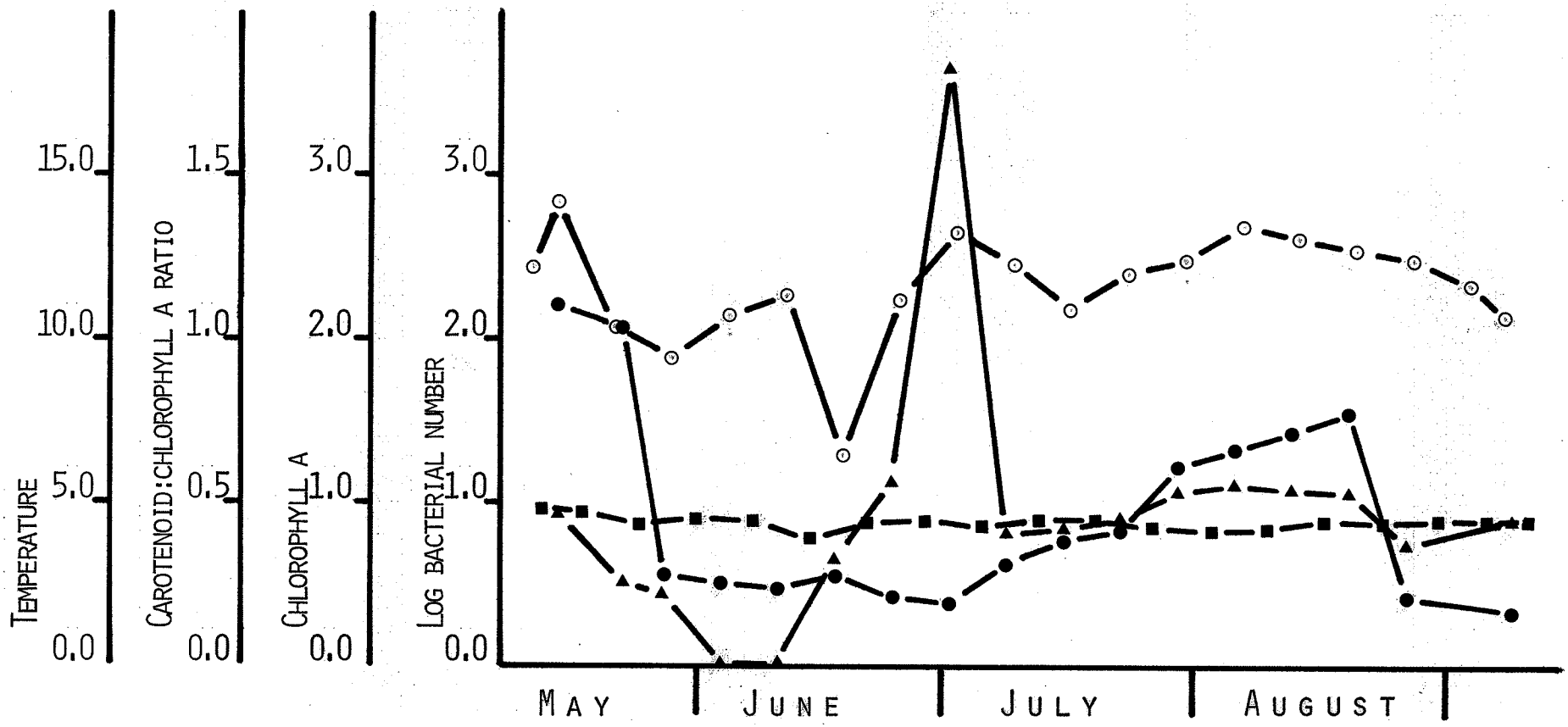

FIG.8: A COMPARISON OF 4 PARAMETERS FOR 25 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

- CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
- CHLOROPHYLL A (UG./L.)
- CAROTENOID:CHLOROPHYLL A RATIO
- TEMPERATURE (°C)

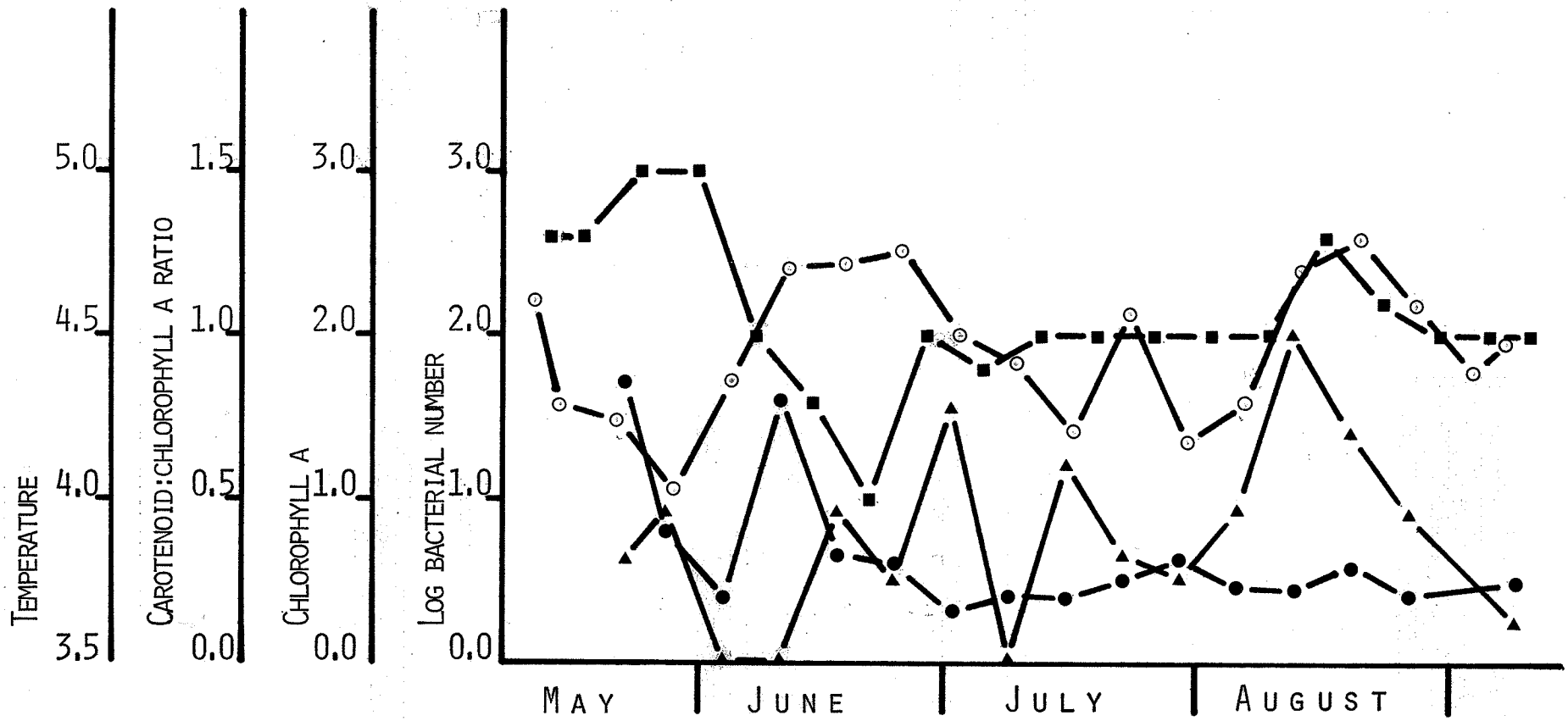

FIG.9: A COMPARISON OF 4 PARAMETERS FOR 30 METERS, 1972, IN WEST BLUE LAKE AT STATION 2.

○—○ CYTOPHAGA PLATE COUNTS (LOG NO./ML.)
●—● CHLOROPHYLL A (UG./L.)
▲—▲ CAROTENOID:CHLOROPHYLL A RATIO
■—■ TEMPERATURE (°C)

Pigment concentrations did not appear to fluctuate as widely as did Cytophaga agar plate counts and as a result interpolation in the weekly sampling intervals probably did not omit any significant concentration changes.

The seasonal chlorophyll a pattern was very similar for 0 to 5 meters (Figures 1-4). The concentration dropped rapidly (at one meter an initial rise and then a drop) till the end of May and then rose gradually peaking in early July and falling to a minimum in late August. By early September the concentration had again increased significantly.

Seven meters was similar to the above pattern, but fluctuated more dramatically (Figure 5).

The remaining 4 depths (Figures 6-9) all produced individual patterns.

At 12 meters following the ubiquitous May decrease, there occurred some weekly oscillations combined with a gradual decline until the September increase. The latter was common to 0 through 17 meters. At 25 and 30 meters at this time the chlorophyll a levels remained constant.

At 17 meters a unique and very pronounced chlorophyll a peak occurred towards the end of July. The pigment concentration virtually tripled and dropped back in a time period of 7 weeks.

At 25 meters a similar, but less intensive peak occurred from late July to late August, that is delayed relative to 17 meters.

At 30 meters close to the bottom of West Blue Lake there was a relatively constant pigment concentration with the exception of a minor spring fall of chlorophyll a and a significant peak in early June.

Although like the other parameters, the carotenoid:chlorophyll a

ratio varied remarkably from depth to depth, a number of similarities between depths did exist.

Generally from 0 to 12 meters the ratio tended to increase slightly - throughout the sampling season. From 0 to 5 meters a discrete increase occurred about July 24th. The most distinctive feature of the ratio common for 0 to 5 meters was the peak of late August (Figures 1-4). Otherwise the depths varied. The pairs 0 and 3 meters, and 1 and 5 meters are more similar than are adjacent depths. This phenomenon is not unique to ratio data, but also occurs, for example, with the bacterial peak of August 14th at 0 and 3 meters.

As a result of a simultaneous zero chlorophyll a concentration, and a peak in carotenoids in early June at 7 meters, the ratio at this time was infinite.

A common feature of 12 to 30 meters were the relatively low carotenoid values occurring about June 11th, and as a result the pigment ratio was low. On June 11th for 25 and 30 meter samples, the pigment ratio was zero.

The depth of 12 meters was uniquely stable with respect to the pigment ratio. The dynamics of the ratio for 17 and 25 meters were similar, although the latter were more pronounced. The ratio oscillations of 30 meters were quite distinct, but may not be of much significance considering the low pigment concentrations.

Due to the stratified state of the lake, temperature is a parameter which was of most significance in the epilimnion and metalimnion.

The seasonal temperature pattern for the epilimnial depths of 0 to 5 meters were similar. The depth of 7 meters is less similar. With the exception of the mid June peak, the temperature increased gradually

until late August at which time the pattern became similar to the epilimnial depths. The 12 meter pattern consisted of a gradual rise in temperature throughout the season that ranged from 4.2°C to 7.8°C.

The depth of 30 meters unlike 17 and 25 meters did fluctuate in temperature. Although the absolute changes were small, they may be significant.

Table 10: The means and standard deviations of Cytophaga agar plate counts, chlorophyll a, carotenoid:chlorophyll a ratio and temperature. The days of the year used in the analysis (Appendix 1) included 136 - 241 and 252 with N = 17. Data from 1972, West Blue Lake.

Depth	Cytophaga agar No./ml.		Chlorophyll a ug/l		Carot:chl a ratio		Temperature °C	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
0	379	502	1.13	0.49	0.63	0.24	16.96	2.94
1	397	457	1.13	0.48	0.65	0.14	16.88	2.94
3	399	500	1.19	0.54	0.70	0.31	16.41	3.13
5	229	178	1.39	0.82	0.65	0.15	15.45	3.59
7	438	669	1.56	0.95	0.62	0.28	12.44	3.98
12	240	236	1.46	0.72	0.56	0.11	6.22	0.95
17	251	186	1.57	0.73	0.42	0.18	4.48	0.18
25	274	176	0.93	0.60	0.47	0.39	4.42	0.18
30	331	165	0.30	0.21	0.32	0.26	4.55	0.24

Table 11: Simple and multiple correlation coefficients of 1972 data for Cytophaga agar plate counts chlorophyll a, carotenoid:chlorophyll a ratio and temperature. Analysis continued from Table 10.

Depth	Cyt to chl a	Cyt to carot:chl a	Cyt to T° C	Chl a to carot:chl a	Chl a to T° C	Carot:chl a to T° C	Multiple "r"
0	-0.088	0.214	-0.056	-0.400	-0.721	0.434	0.310
1	-0.086	0.217	0.075	-0.614	-0.445	0.295	0.228
3	-0.099	0.100	-0.295	-0.443	-0.731	0.284	0.549
5	0.579	0.356	-0.405	-0.278	-0.767	0.566	0.846
7	0.324	-0.067	0.011	0.011	-0.683	0.197	0.484
12	-0.458	0.029	-0.084	-0.243	-0.334	0.243	0.525
17	0.397	0.333	-0.202	0.263	-0.312	0.210	0.491
25	0.516	0.466	0.182	-0.071	0.212	-0.056	0.727
30	0.083	0.028	-0.434	-0.092	0.116	-0.048	0.454

DISCUSSION

Cytophaga agar plate count fluctuations and possible contributing factors

Cytophaga agar counts will be treated as the object of the 3 possibly interrelated factors; chlorophyll a, the carotenoid:chlorophyll a ratio and temperature.

The most pronounced correlation of any 2 isolated factors was the negative relationship between chlorophyll a and temperature. This relationship was most evident during times of maximum temperature change, particularly spring and fall.

The single factor which graphically appeared most likely as the factor stimulating bacterial peaks was chlorophyll a. Particularly for the epilimnial depths and ignoring the initial exceptional spring drop of chlorophyll a, bacterial numbers peaked as chlorophyll a concentrations were decreasing. Some of the bacterial peaks also coincided with chlorophyll a peaks, a complication that will be discussed in conjunction with the carotenoid:chlorophyll a ratio.

There was no significant direct relationship of temperature and Cytophaga agar plate counts. Biochemically, increased temperatures tend to increase rates of metabolic activity. Indirectly by bacterial uptake of ^{14}C glucose it has been shown (Robinson etal 1973, unpublished) that bacterial numbers are related to temperature.

Temperature changes were related to the carotenoid:chlorophyll a ratio. But as with all attempts at defining carotenoid:chlorophyll a ratios, the relationship was never consistent. Maximal changes in the ratio occurred at times of temperature change, but the relationship was direct or indirect or both occurring within one period of temperature change.

Chlorophyll a was obviously, mathematically speaking, negatively correlated to the carotenoid:chlorophyll a ratio. A decrease in the ratio was associated with renewed vigor and its attendant photosynthesis and biomass increases which would be reflected by chlorophyll a increases, the cause and result of a decreased ratio, for the latter the ratio change being merely a symptom. Reduced vigor and decreased photosynthesis results in chlorophyll a breaking down more rapidly than carotenoids causing an increase in the ratio.

The relationship of bacterial number fluctuations and the carotenoid:chlorophyll a ratio cannot be strictly defined. One complicating factor is the fact that the ratio is symptomatic of both physiology and species composition. Can these 2 causes be distinguished? For instance, in early spring as chlorophyll a was falling rapidly, the ratio was fluctuating and this can be related to known species changes occurring at this time (Cliff M.S. 1972). But even at this time physiological changes were probably also occurring simultaneously.

The phytoplankton are known to excrete products (Lund 1965) of importance to the bacterial community. These extracts can be stimulatory or inhibitory. Excretion of soluble organic substrates is probably continuous. The rate of excretion and the nature of the excreted products will be dependent on species composition and physiology. A chlorotic unhealthy community may release soluble organics by senescence and autolysis (relatively high carotenoid:chlorophyll a ratio). A healthy phytoplankton community (relatively low carotenoid:chlorophyll a ratio) may photosynthesize in excess of growth and reproduction, an excess which must appear as excreted products.

That the ratio is related to bacterial number fluctuations can be

assum ed but the relationship cannot be defined to be applicable to the majority of situations.

Examining the data for the entire 1972 season, the carotenoid: chlorophyll a ratio appeared to be positively correlated to bacterial numbers on the basis that the ratio increased, particularly for the upper portion of the water column, throughout the season. Bacterial fluctuations, if smoothed out, showed a similar increase towards the season's end.

More specifically and quantitatively a relationship could be shown between bacterial numbers and the carotenoid:chlorophyll a ratios that occurred late in August and early September. Peaks occurred from 0 to 5 meters in the ratio, but of varying amplitude from depth to depth. These amplitudes were related to subsequent bacterial number changes.

At 0 and 1 meters there was a direct positive correlation of the ratio peak and Cytophaga agar counts at that time. The ratio peak at 0 meters had a larger amplitude, and its decline caused a September increase in bacterial counts, while at 1 meter numbers actually dropped slightly, following the ratio peak. A similarity existed between 1 and 5 meters. But at 3 meters, where the ratio attained the largest amplitude, the ratio decline was succeeded by a bacterial increase significantly larger than at any of the other depths.

The following are examples of the major Cytophaga agar plate count peaks with brief explanations and descriptions of the possible contributing variables.

Preceeding this is a discussion of a common feature of most of the depths, the early spring drop of chlorophyll a, its consequences and possible explanation. The phenomena that occurred at this time were probably related to the aftermath of the spring overturn. The resultant

mixing introduced a renewed supply of nutrients which may have been subsequently exhausted by the early spring bloom of both algae and bacteria. This was followed by some self annihilative process. This entire process occurred at almost all depths. Even though both bacterial numbers and chlorophyll a drop off rapidly, no comparable succeeding bacterial peak occurred. Assuming that the die-off resulted in an abundant supply of soluble organics, a bacterial peak might have been expected. One explanation might be some sort of inhibitory effect. Another being a species composition change rather than solely a chlorophyll a (biomass) decrease. Evidence for this occurrence exists (Cliff M.S. 1972).

At 0 meters the August Cytophaga agar plate count peak occurred after a chlorophyll a drop, during a slight temperature depression and as it was initiated, a slight pigment ratio rise and fall occurred.

At 1 meter the relatively high early July and early August bacterial peaks could be explained by decreasing chlorophyll a levels. Although the pigment ratio was quite constant it tended to be positively correlated to Cytophaga agar counts. The July 10th Cytophaga agar count peak did not occur to the same extent at any other depth. The reason may be that despite all 4 epilimnial depths having had similar chlorophyll a seasonal profiles only 1 meter possessed a chlorophyll a drop at this time.

The August peak of 3 meters occurred as chlorophyll a levels dropped. Both temperature and pigment ratios formed slight troughs at this time.

Although relatively minor, the bacterial peaks at 5 meters appeared to occur in response to chlorophyll a drops even though these may have been small. This depth was quite stable, for even pigment ratio fluc-

tuations were slight, which provides an explanation for little bacterial change with respect to bacterial number fluctuations.

While 5 meters was in the epilimnion, 7 meters was below the initiation of the thermocline for the major part of the sampling season, that is, following the initiation of the thermocline. Although 7 meters may have been quite similar to other depths in certain respects, it was completely contradictory in others. Except for the late August bacterial Cytophaga counts were not only graphically positively correlated to chlorophyll a, but computer multiple regression analysis support this. Even the major bacterial peak of early July corresponded to a chlorophyll a peak. The late August peak at 7 meters occurred concomitantly with a temperature rise as well as a drop in the pigment ratio and was preceeded by a decrease in the chlorophyll a concentration. As the pigment ratio rose again, bacterial numbers dropped.

At 12 meters (below the thermocline) there was good agreement between chlorophyll a decreases and Cytophaga agar count increases. Temperature and the carotenoid:chlorophyll a ratio appeared to be of little significance although the latter was at times positively correlated to Cytophaga agar counts. The negative correlation of chlorophyll a and Cytophaga agar numbers was particularly good immediately following the spring drop.

At 17 meters an unprecedented midsummer chlorophyll a peak occurred which was difficult to explain as was its lack of effect relatively speaking on the Cytophaga agar counts. Apparently the rise of chlorophyll a caused a decrease and a plateau of Cytophaga agar numbers, but as chlorophyll a decreased a major Cytophaga agar count peak was expected, but only a very small peak occurred which immediately subsided.

Although Cytophaga agar counts decreased, tryptic soy agar and succinate agar counts did rise significantly. The different media may have been supporting quite different populations. Cytophaga agar counts did correlate positively with the carotenoid:chlorophyll a ratio.

The similar but delayed chlorophyll a peak at 25 meters occurred during August and similarly coincided with decreasing Cytophaga agar counts. As at 17 meters tryptic soy agar and succinate agar counts increased after the chlorophyll a peak. The bacterial peak (Cytophaga agar) occurring from June to July appeared to occur in response to a phenomenally high pigment ratio and possibly a slight chlorophyll a decrease. Otherwise Cytophaga agar counts tended to be positively correlated with the pigment ratio and chlorophyll a levels. The pigment ratio appeared to be the prime determining factor for bacterial number changes at this depth.

At both 17 and 25 meters in early June as the pigment ratio became zero bacterial numbers were high and began to drop as the ratio began to increase. And again at both depths as the ratio reached its peak, bacterial numbers also peaked in early July. For both 17 and 25 meters bacterial number increases had a tendency to succeed carotenoid:chlorophyll a ratio peaks, that is, the Cytophaga agar count peaks were positively correlated but slightly delayed or out of phase.

If at 30 meters the Cytophaga agar counts were arbitrarily divided into 2 peaks, the first in June and the second in August, the first may have occurred in response to the spring drop of chlorophyll a and the subsequent peak was coincidental with a zero pigment ratio. The second or August bacterial peak coincided with a pigment ratio peak.

The seasonal temperature pattern, the chlorophyll a pattern and the homogeneity of the temperature depth profile indicated that mixing

of the epilimnion was virtually instantaneous. Although there were slight differences particularly of chlorophyll a for the 4 epilimnial depths, the bacterial numbers might have been expected to fluctuate similarly and in phase for these depths. Although similarities of bacterial counts did exist between depths, major exceptions did occur such as the July 10th peak at one meter. This exception can be explained by a corresponding chlorophyll a difference.

Similarities in the bacterial number patterns might have been expected as a result of changes in number initiated at one point and by mixing and rapid reproduction, spreading to all epilimnial depths within a sampling interval (7 days). In spite of this numbers would still differ from depth to depth due to time lags, gravitational drop during periods of decreased turbulence, and depth gradients such as light penetration, a slight temperature profile and oxygen concentration gradients.

An example, in the hypolimnion, of what might have been gravitational drop were the 2 major chlorophyll a peaks that occurred at 17 and 25 meters. The peaks were out of phase as though caused by a time lag due to sinking from 17 to 25 meters.

If bacterial changes were initiated and did spread to adjacent depths it is quite possible that the weekly sampling interval may have missed a peak at one or more depths. These peaks may have been formed rapidly and subsided in the interval between two sampling dates. Other data particularly chlorophyll a may predict their occurrence.

It was found that graphical analysis was preferable to multiple regression by computer, the latter having a number of drawbacks. The results are too easily biased by single exceptional data pairs. The

sampling dates for the various parameters do not coincide, data cannot be validly interpolated in many cases, but for computer analysis the data was treated as though all the parameters were sampled on one day (for explanation of data grouping see Appendix 1). But considering that it is pigment and temperature data that has been grouped to conform to bacterial sampling dates, the grouping is probably reasonably valid. This is due to the data forming reasonably smooth curves as opposed to the fluctuations of bacterial counts. The various parameters being compared must be independent or the end results will be positively biased.

In graphical analysis irregularities and exceptions can be rationalized on the grounds of there being influencing factors other than those being discussed, but the computer is objective and for ecological work likely to be impractical from this point of view, frequently distorting obvious relationships.

Of the multiple regression results 4 interesting simple correlations were quite similar for 0 to 5 meters. Cytophaga agar counts were positively correlated with the pigment ratio, but not very significantly. Chlorophyll a was negatively correlated with both the pigment ratio and temperature. The pigment ratio was positively correlated with temperature. Unlike graphical analysis, the chlorophyll a to Cytophaga agar count relationship did not show up here except at 5 and 7 meters where the correlation coefficient was positive. At 7 meters the carotenoid: chlorophyll a ratio was not significantly correlated with any of the other parameters. For 12 meters the chlorophyll a, the carotenoid: chlorophyll a ratio and the temperature correlations were similar to those of the epilimnial depths. The only significant Cytophaga agar count relationship was the negative correlation with chlorophyll a.

At 17 meters only the temperature to chlorophyll a and to the carotenoid:chlorophyll a ratio relationships were retained as compared to the epilimnial depths. At both 17 and 25 meters Cytophaga agar counts were positively correlated with chlorophyll a and the carotenoid:chlorophyll a ratio.

The only significant correlation at 30 meters was the negative correlation of Cytophaga agar counts and temperature. Due to the absence of many other disturbing influences at this depth the slight temperature fluctuations that did occur may be quite significant. It is strange from the biochemical point of view that here and at some of the other depths, that temperature should correlate negatively with bacterial numbers rather than positively. Increasing temperature within reason should increase biochemical activity and hence metabolism, growth and reproduction.

RESULTS

Heterotrophy

Although Cytophaga agar plate counts do enumerate heterotrophic organisms, other media and procedures can be used to obtain more specific information such as the potential of single acids as substrates. This information was obtained by direct inoculation onto succinate agar and by replica plating from Cytophaga agar onto succinate agar and 10 other substrates.

Although overall correlations of direct plate counts on succinate agar and Cytophaga agar were not always good, at particular times and depths the correlations were excellent. These instances occurred during bacterial peaks, especially at the depths close to the lake's surface. The correlations of the media were not as good during periods of lower counts.

The major difference between Cytophaga agar and directly inoculated succinate agar plate counts occurred from 0 - 7 meters during the spring drop of bacterial counts and chlorophyll a. As might be expected from the discussion of the Cytophaga agar count, chlorophyll a relationship, bacterial counts were expected to increase. Succinate agar counts do increase, but Cytophaga agar counts do not.

The directly inoculated succinate agar plate counts are presented in Figures 10, 11, 12, 13, 14. A comparison of 0 - 7 meters revealed common bacterial peaks that occurred in late May, early July, the middle of August and at the end of the sampling season. Periods of very low or zero succinate direct plate counts occurred from 0 - 7 meters at the beginning of the sampling season, during late May to early June and at the end of July. The remaining depths of 12 - 30 meters are quite

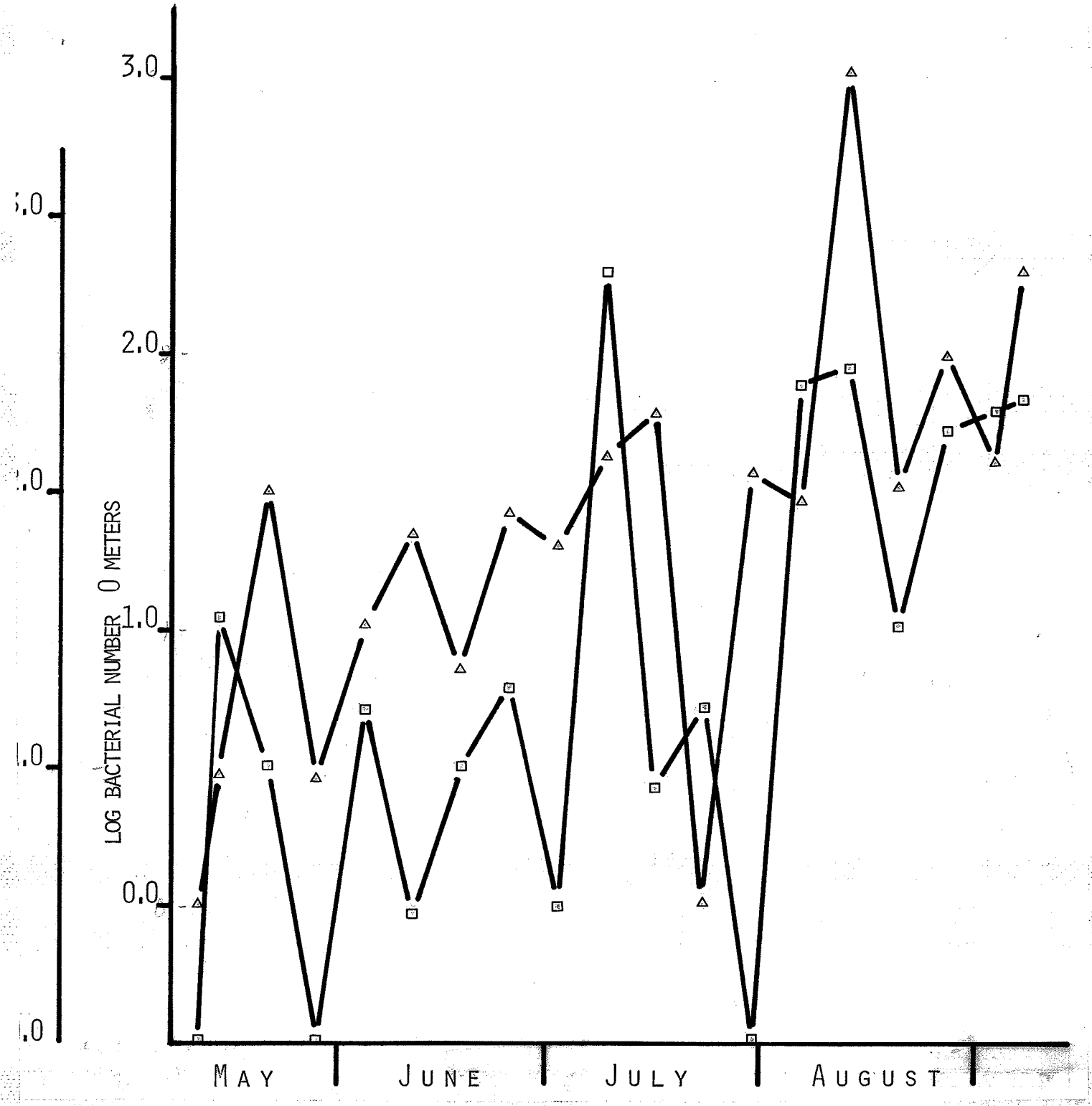

FIG.10: A COMPARISON OF DIRECT PLATE SUCCINATE COUNTS FOR 0 AND 1 METERS AT STATION 2, WEST BLUE LAKE, 1972. (LOG NO./ML.)

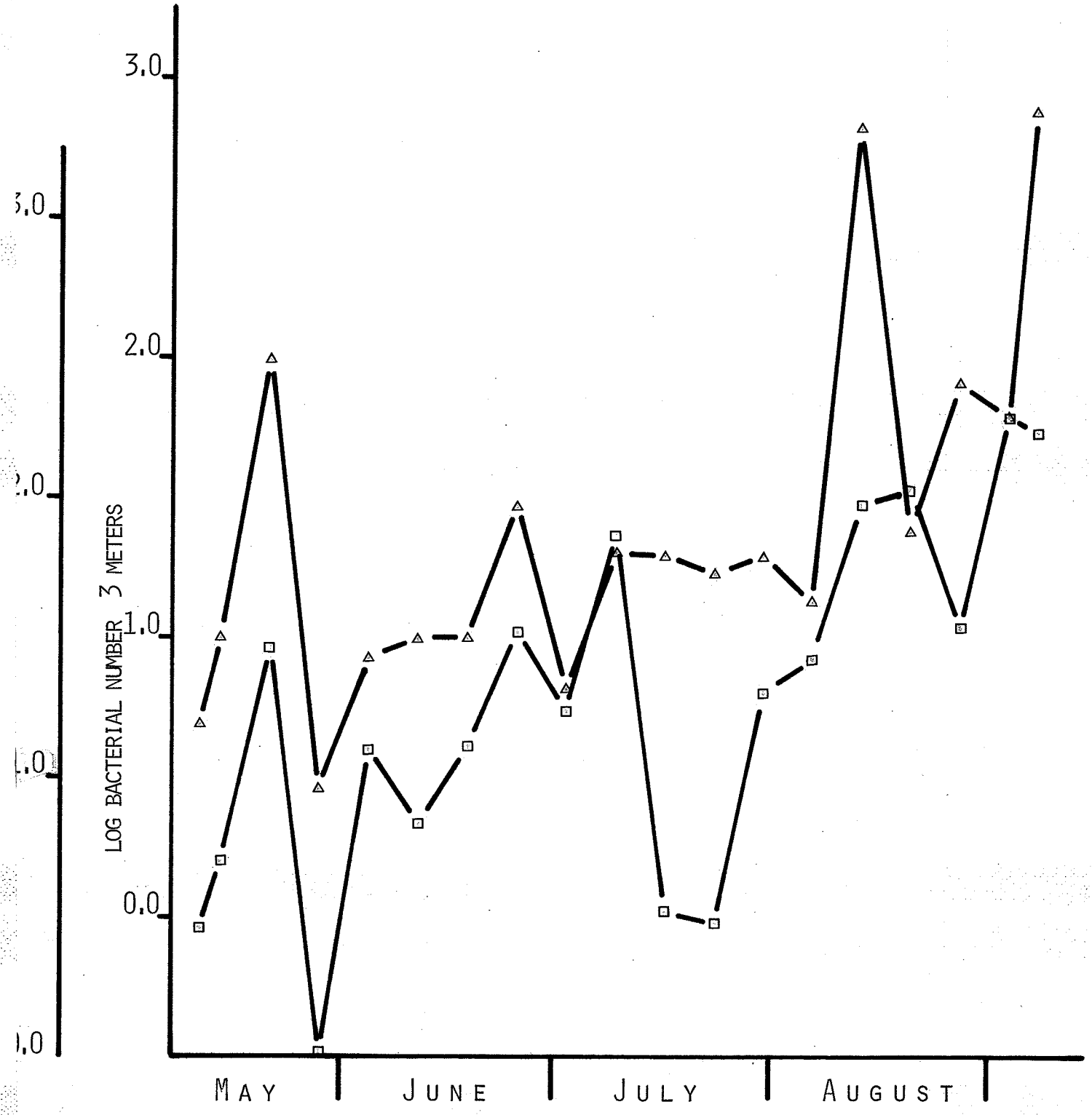

FIG.11: A COMPARISON OF DIRECT SUCCINATE PLATE COUNTS FOR 3 AND 5 METERS AT STATION 2, WEST BLUE LAKE, 1972. (LOG NO./ML.)

△—△ 3 METERS
□—□ 5 METERS

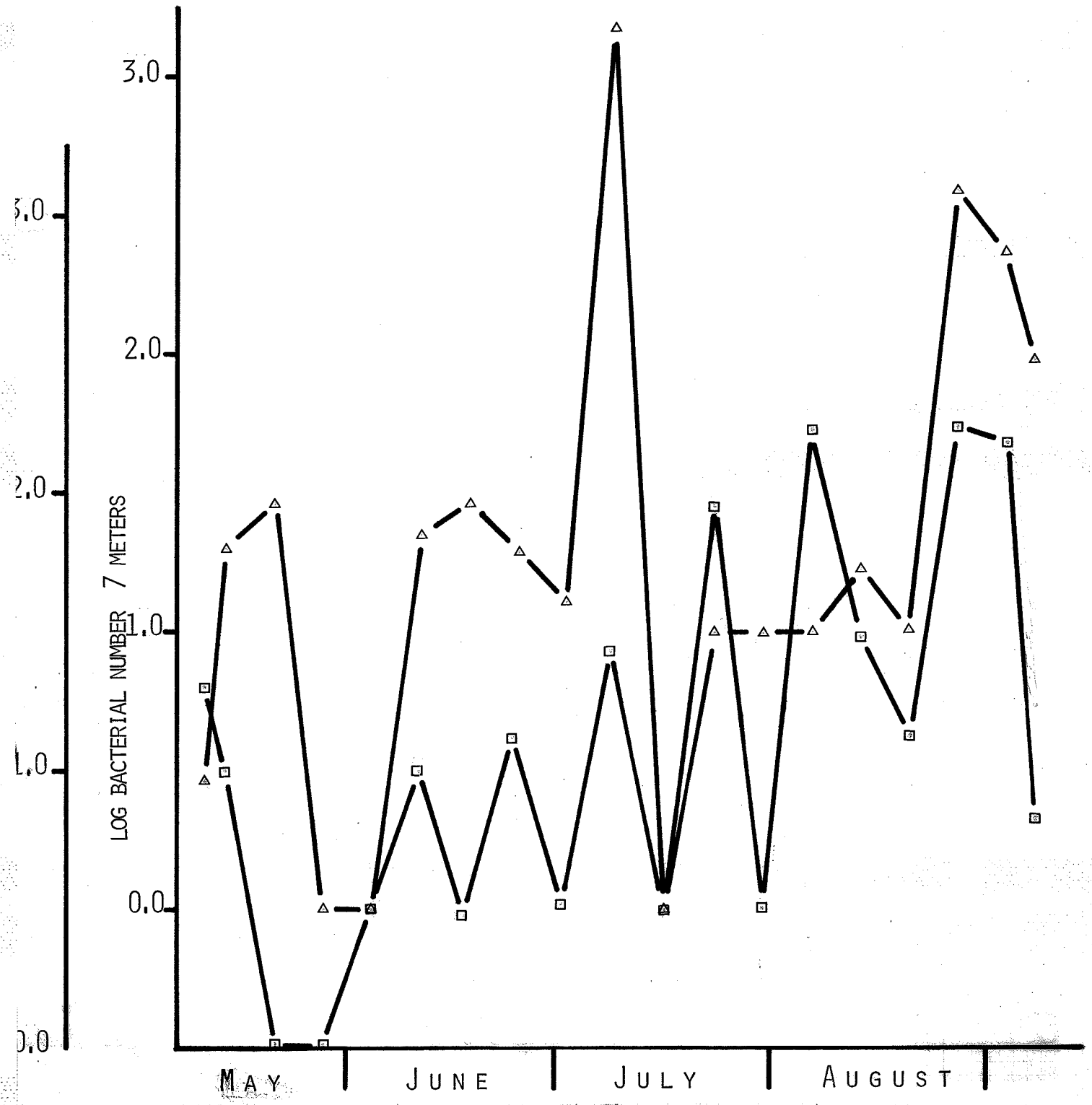

FIG.12: A COMPARISON OF DIRECT SUCCINATE PLATE COUNTS FOR 7 AND 12 METERS AT STATION 2, WEST BLUE LAKE, 1972. (LOG NO./ML.)

△——△ 7 METERS
□——□ 12 METERS

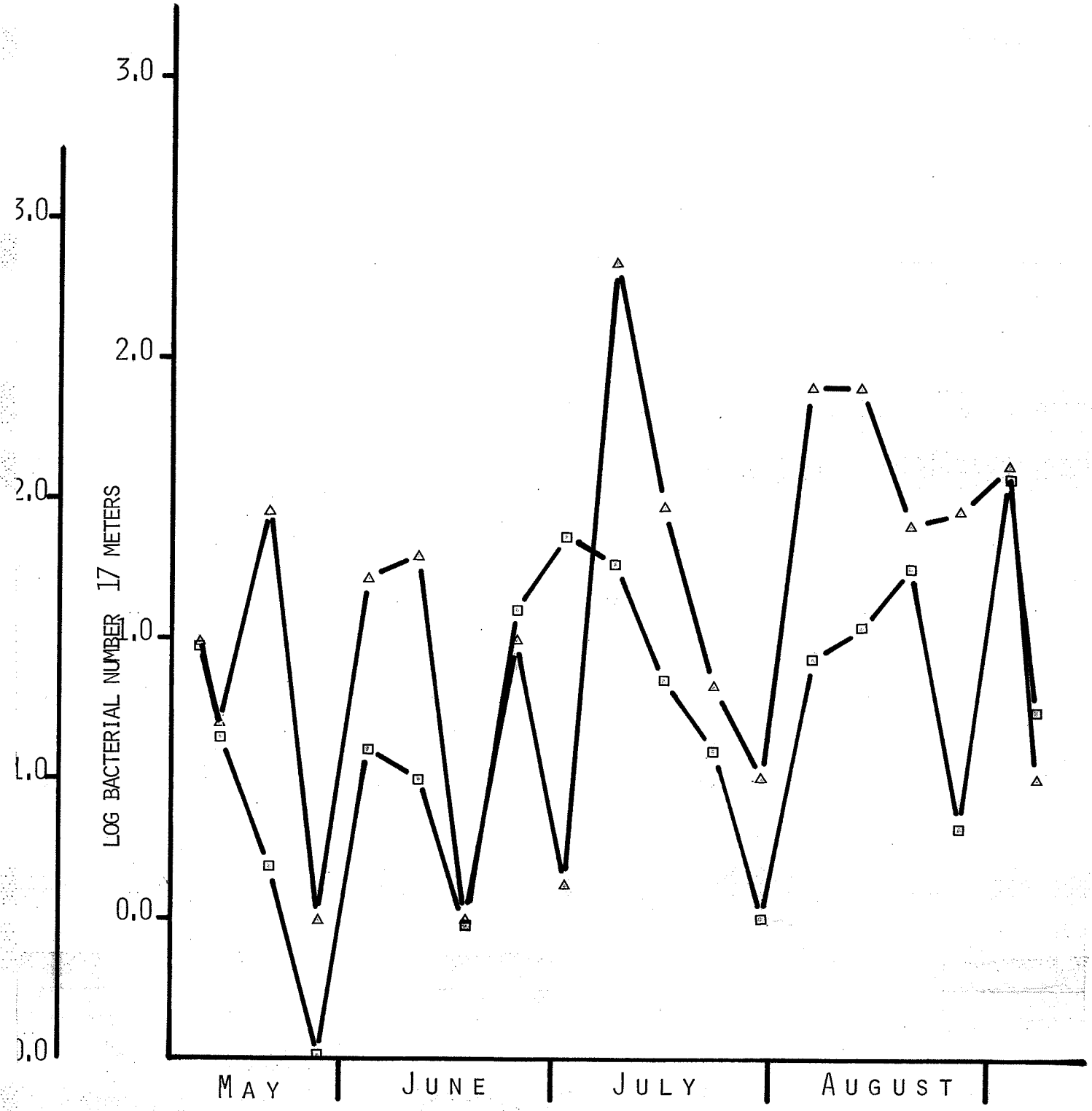

FIG.13: A COMPARISON OF DIRECT SUCCINATE PLATE COUNTS FOR 17 AND 25 METERS AT STATION 2, WEST BLUE LAKE, 1972. (LOG NO./ML.)

△—△ 17 METERS
□—□ 25 METERS

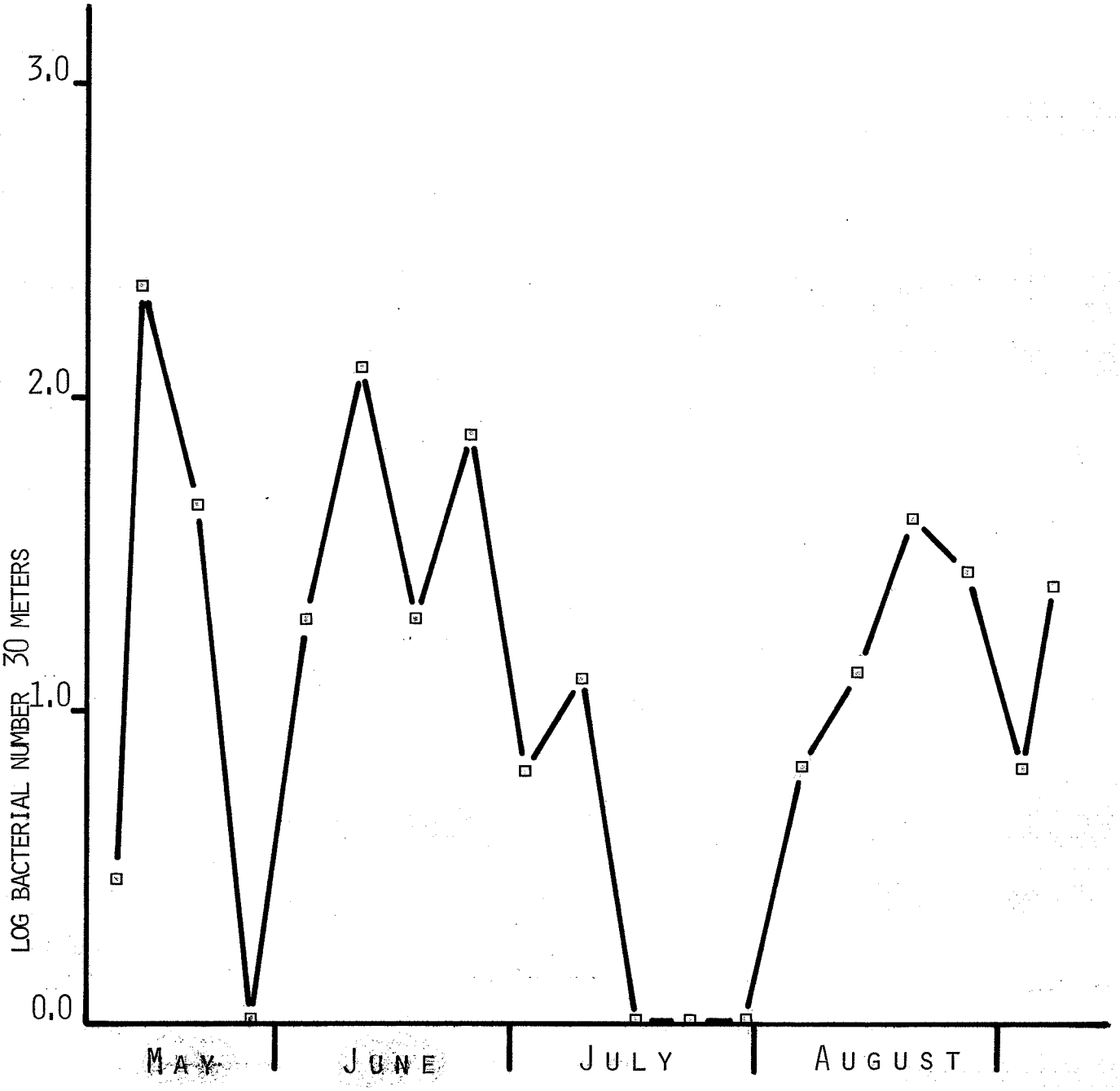

FIG.14: DIRECT SUCCINATE PLATE COUNTS FOR 30 METERS AT STATION 2, WEST BLUE LAKE, 1972. (LOG NO./ML.)

□ —— □ 30 METERS

dissimilar from each other.

The 5 depths used for replica plating produced counts that correlated much better with Cytophaga agar than did the directly inoculated succinate counts. The calculated numbers per milliliter of the former were usually much higher (Figures 15, 16).

Directly inoculated succinate plate counts were compared with replicated succinate counts by regression analysis to analyze for the phenomenon of plating efficiency (Table 12). Graphically the phenomenon is shown for the sums of 0, 3, 7, 17 and 30 meters (Figure 17).

Just as Cytophaga agar plate counts were related to chlorophyll a, carotenoid:chlorophyll a ratio and temperature data, directly inoculated succinate counts, replicated succinate counts and the sum of these for the 5 replica plating depths can be compared and analyzed given the contributing factors.

The seasonal pattern of temperature of the mean of the 5 replica plating depths is virtually identical to any one of the seasonal pattern of temperature of one of the epilimnial depths alone. The carotenoid: chlorophyll a ratio has a tendency to increase as the season progresses. The chlorophyll a curve is distinguished by the spring drop, a peak followed by another drop, another smaller peak and a gradual drop until the end of August just prior to the September rise in pigment concentration (Figure 18).

It can be seen that the 2 major bacterial peaks occurred following the 2 chlorophyll a decreases (Figures 17, 18). During August the bacterial counts were positively correlated to the carotenoid:chlorophyll a ratio. The summed data of the 5 replica plating depths substantiates the negative correlation of chlorophyll a and temperature.

The replicated succinate agar counts for each of the 5 depths

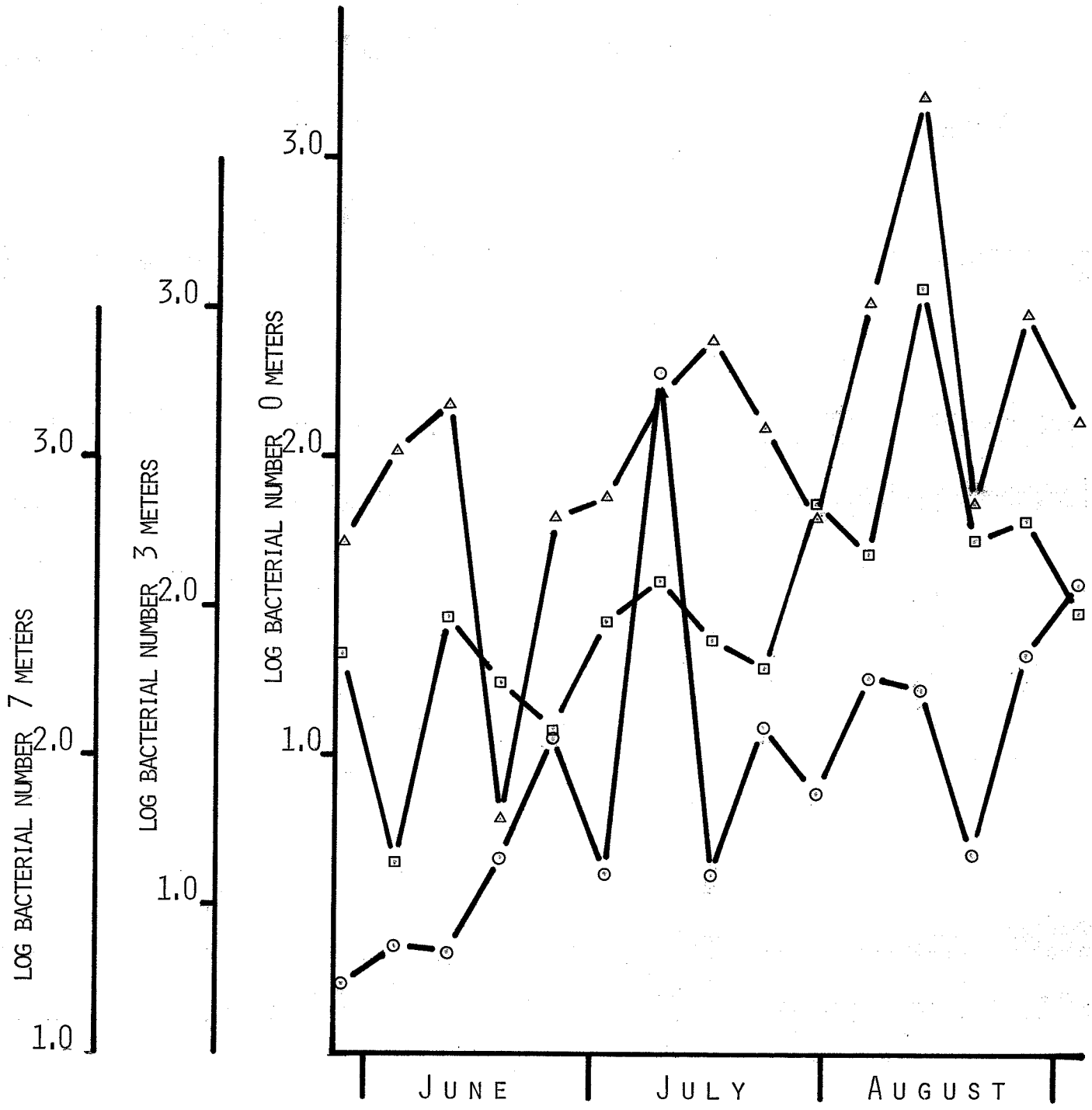

FIG.15: A COMPARISON OF REPLICA PLATING SUCCINATE COUNTS FOR 0, 3 AND 7 METERS AT STATION 2 WEST BLUE LAKE, 1972. (LOG NO./ML.)

△—△ 0 METERS
□—□ 3 METERS
○—○ 7 METERS

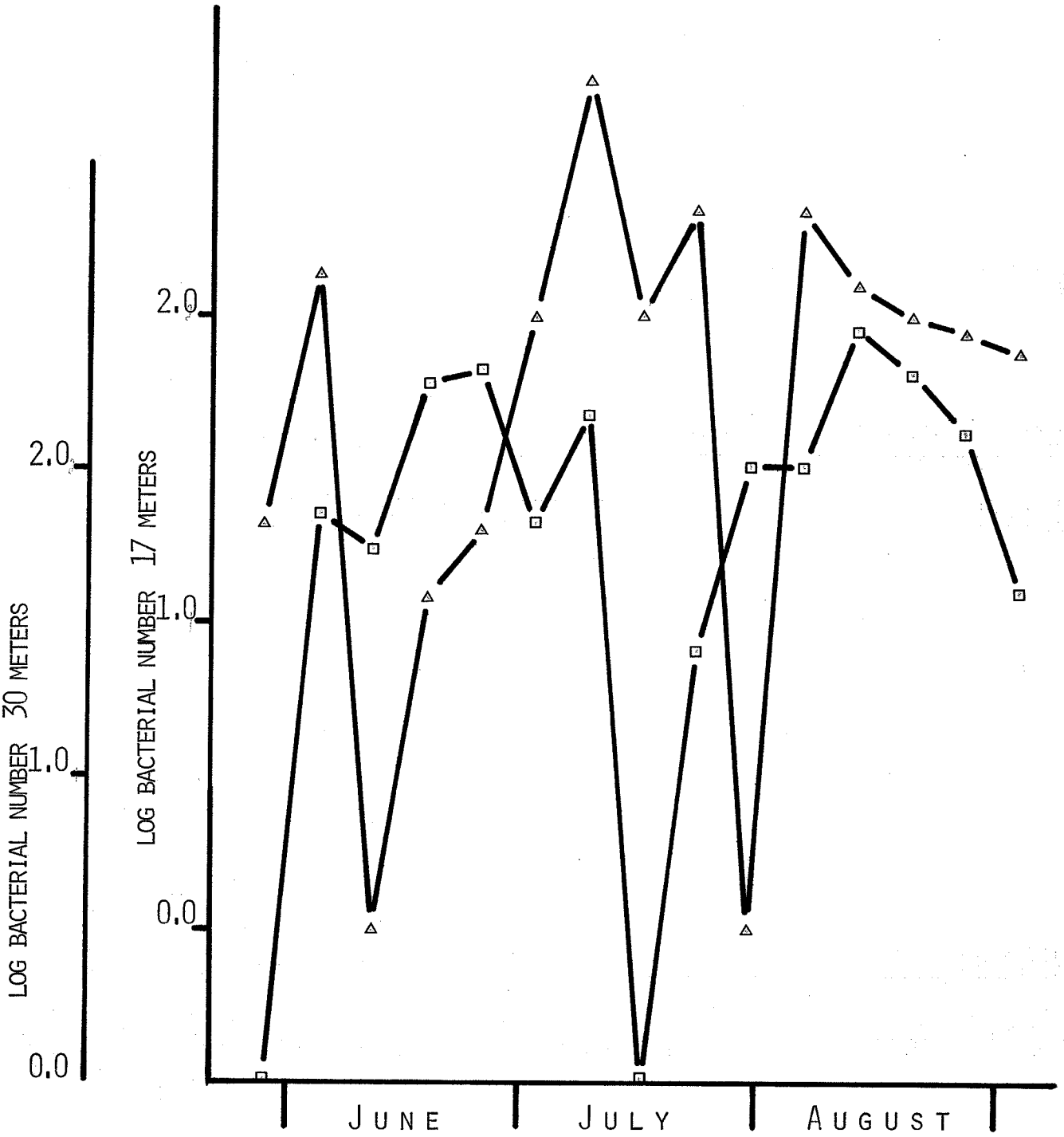

FIG.16: A COMPARISON OF REPLICA PLATING SUCCINATE COUNTS FOR 17 AND 30 METERS AT STATION 2, WEST BLUE LAKE, 1972. (LOG NO./ML.)

△——△ 17 METERS
□——□ 30 METERS

Table 12: A comparison of the means of two methods of determining succinate utilization ability including the ratios of the two and the results of a regression of the two. The two are direct succinate plate counts and replica plating succinate plate counts from 1972, West Blue Lake (numbers per milliliter).

Depth	Mean direct Suc counts	Mean replica Suc counts	Ratio of Replica:direct	Correlation coefficient	Regression "B" value	Regression "Y" intercept
0	98	233	2.376	0.980	0.653	-54.0
3	66	175	2.650	0.981	0.589	-36.9
7	152	222	1.462	0.980	0.800	-25.7
17	39	122	3.083	0.895	0.348	-2.9
30	25	111	4.453	0.199	0.085	15.5

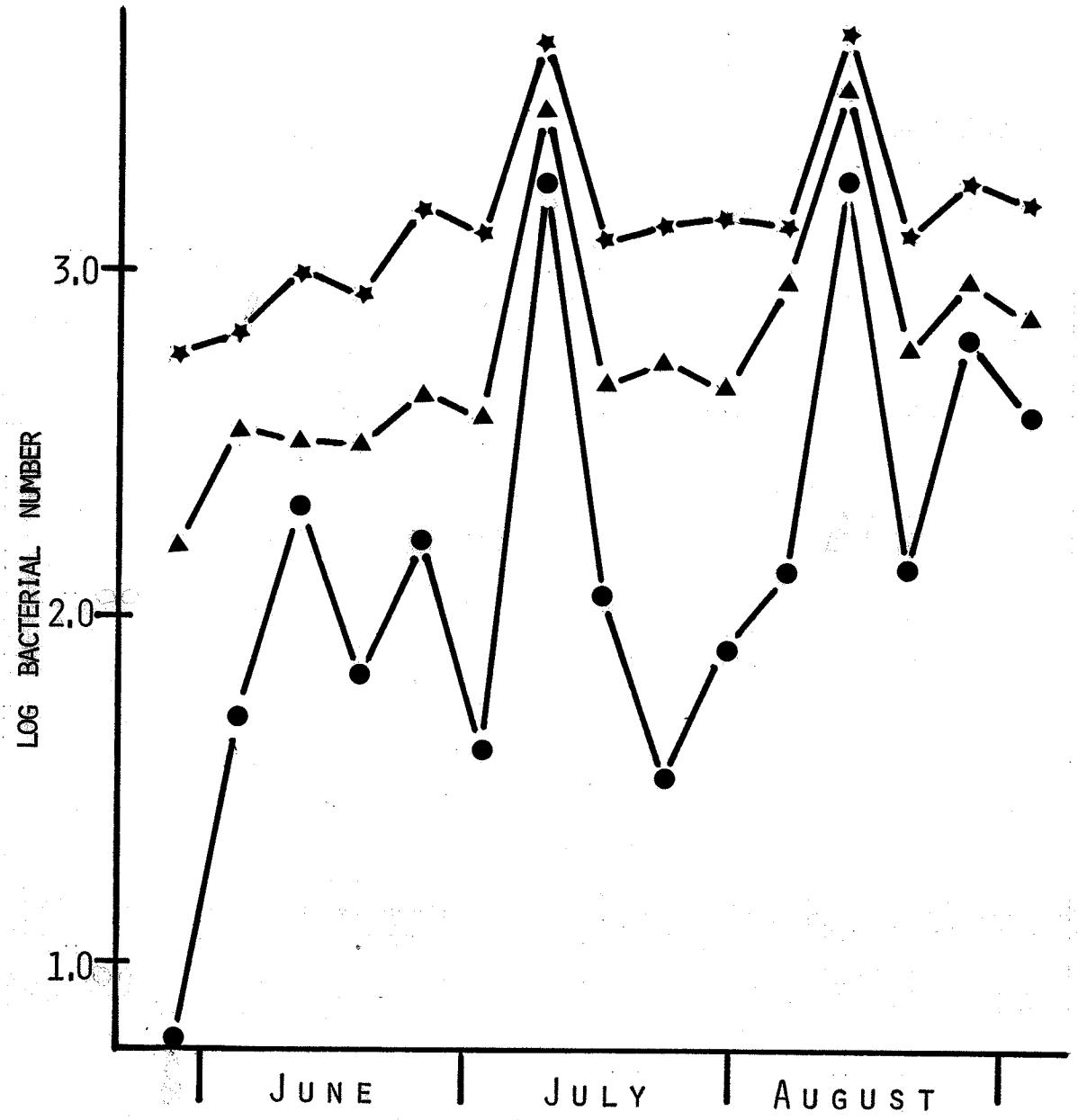

FIG.17: A COMPARISON OF TWO MEANS OF SUBSTRATE PREFERENCE DETERMINATION (1972)

★—★ CYTOPHAGA-INITIAL INOCULATION

▲—▲ SUCCINATE-REPLICATED FROM CYTOPHAGA

●—● SUCCINATE-INITIAL INOCULATION

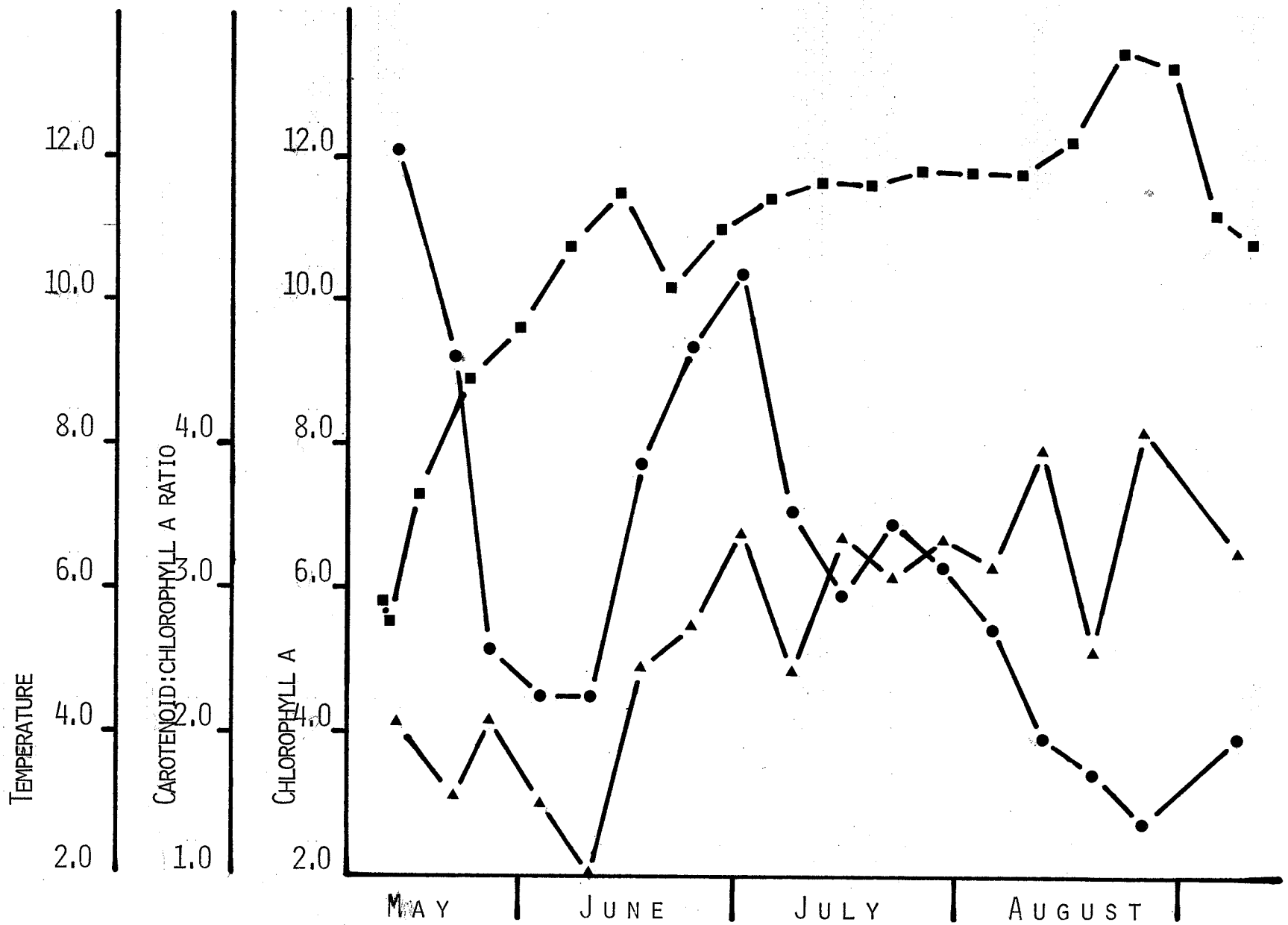

FIG.18: A COMPARISON OF COMBINED DATA OF 0, 3, 7, 17 AND 30 METERS FOR 1972, FROM STATION 2, WEST BLUE LAKE.

● —— ● CHLOROPHYLL A (UG./L.)
▲ —— ▲ CAROTENOID:CHLOROPHYLL A RATIO
■ —— ■ TEMPERATURE (°C)

(Figures 15, 16) were also related to temperature and pigment data. Since generally the correlations with Cytophaga agar counts were excellent, the relationships would be expected to be the same as for the relationship between Cytophaga agar counts and temperature and pigment. One major exception occurred during the chlorophyll a peak at 17 meters. Cytophaga agar counts were unaffected by the chlorophyll a peak while replicated succinate agar counts dropped almost to zero at the peak's maximum and then rose significantly as chlorophyll a levels subsided. This same exception occurred with the directly inoculated succinate counts (Figures 10 - 14). For 0 through 7 meters direct succinate counts rose at the beginning of the sampling season as chlorophyll a levels and Cytophaga agar counts dropped.

The remaining replica plating media correlated with replicated succinate counts and Cytophaga agar plate counts (Figures 19, 20). The 2 major exceptions were lactate and glycolate. The mean proportion of the colonies on Cytophaga agar of the 9 organic acids varied from 0.10 - 0.35 with a mean of 0.20. From the mean data (0, 3, 7, 17, 30 meters) of the replica plating results the proportion order (proportion of cytophaga on a particular substrate) of the 11 substrates was as follows; glutamate, glucose, succinate, malate, fumarate, pyruvate, acetate, citrate, tartarate, glycolate and lactate (Table 13).

The relationship of glutamate, glucose and succinate in the sequence varied slightly from depth to depth (Table 13). Although on a quantitative basis the substrates were significantly different, the seasonal fluctuations of each correlated almost perfectly. Exceptions to this correlation occurred at 30 meters and in cases where the mean counts of the substrate were low, especially for glycolate and lactate.

Table 13: Mean of 11 replica plating media plate counts from the 5th of May to the 4th of September, 1972 with N = 14 at West Blue Lake, Station 2 (Numbers per milliliter).

	Mean of 5 depths	0 meters	3 meters	7 meters	17 meters	30 meters
Glutamate	210	233	217	262	172	169
Glucose	189	263	151	288	136	104
Succinate	182	245	182	237	129	119
Malate	154	201	140	211	121	95
Fumarate	126	192	109	173	103	51
Pyruvate	120	175	107	171	64	85
Acetate	105	151	89	154	71	58
Citrate	73	81	50	134	50	48
Tartarate	24	27	43	41	10	1
Glycolate	17	31	17	28	3	5
Lactate	6	7	2	8	4	10

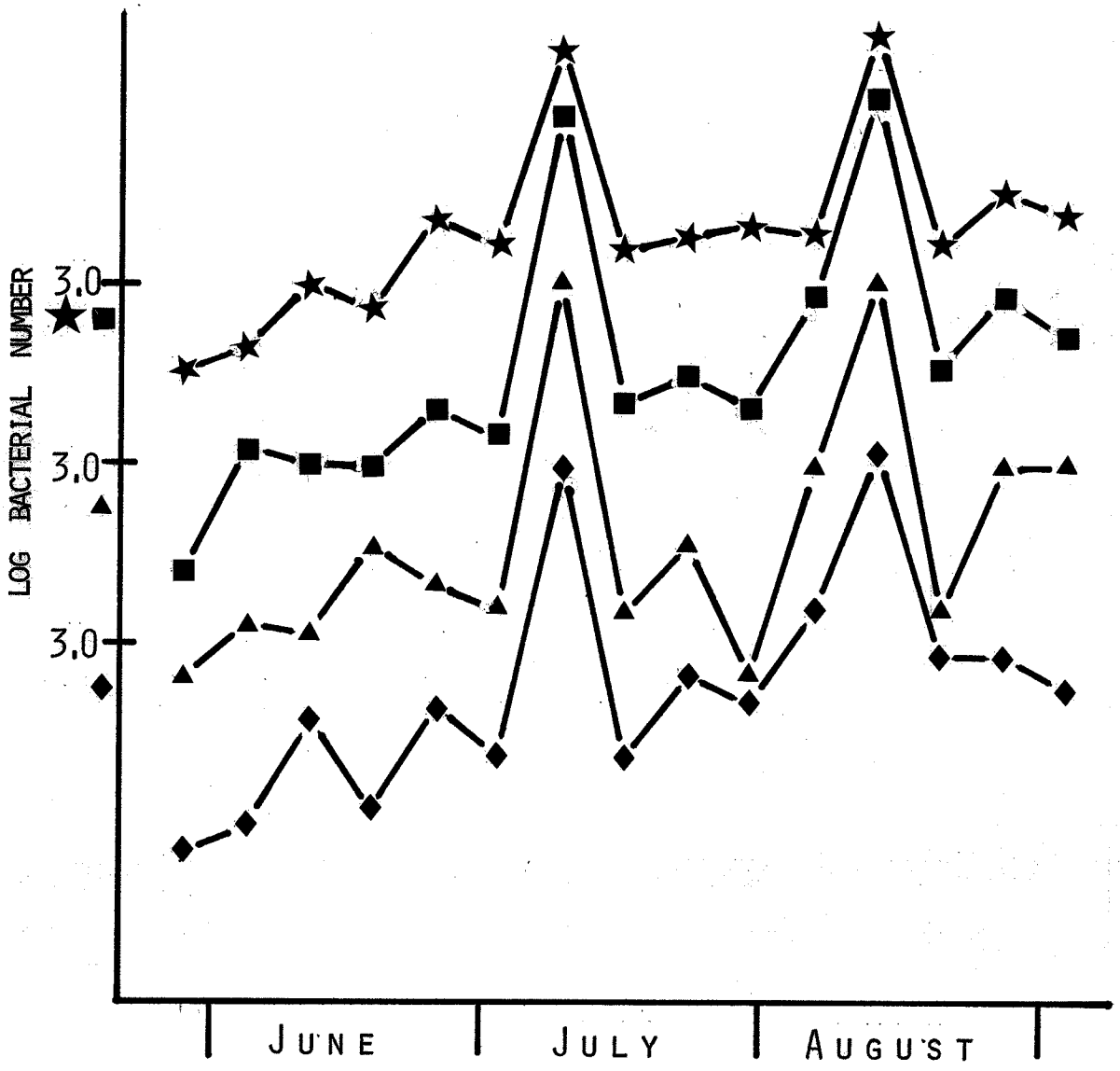

FIG. 19: SEASONAL CHANGES IN THE NUMBER OF BACTERIA CAPABLE OF UTILIZING CERTAIN SUBSTRATES, QUANTIFIED VIA REPLICA PLATING (1972).

★—★ CYTOPHAGA-SOURCE OF REPLICATED PLATES
■—■ SUCCINATE
▲—▲ GLUCOSE
◆—◆ GLUTAMATE

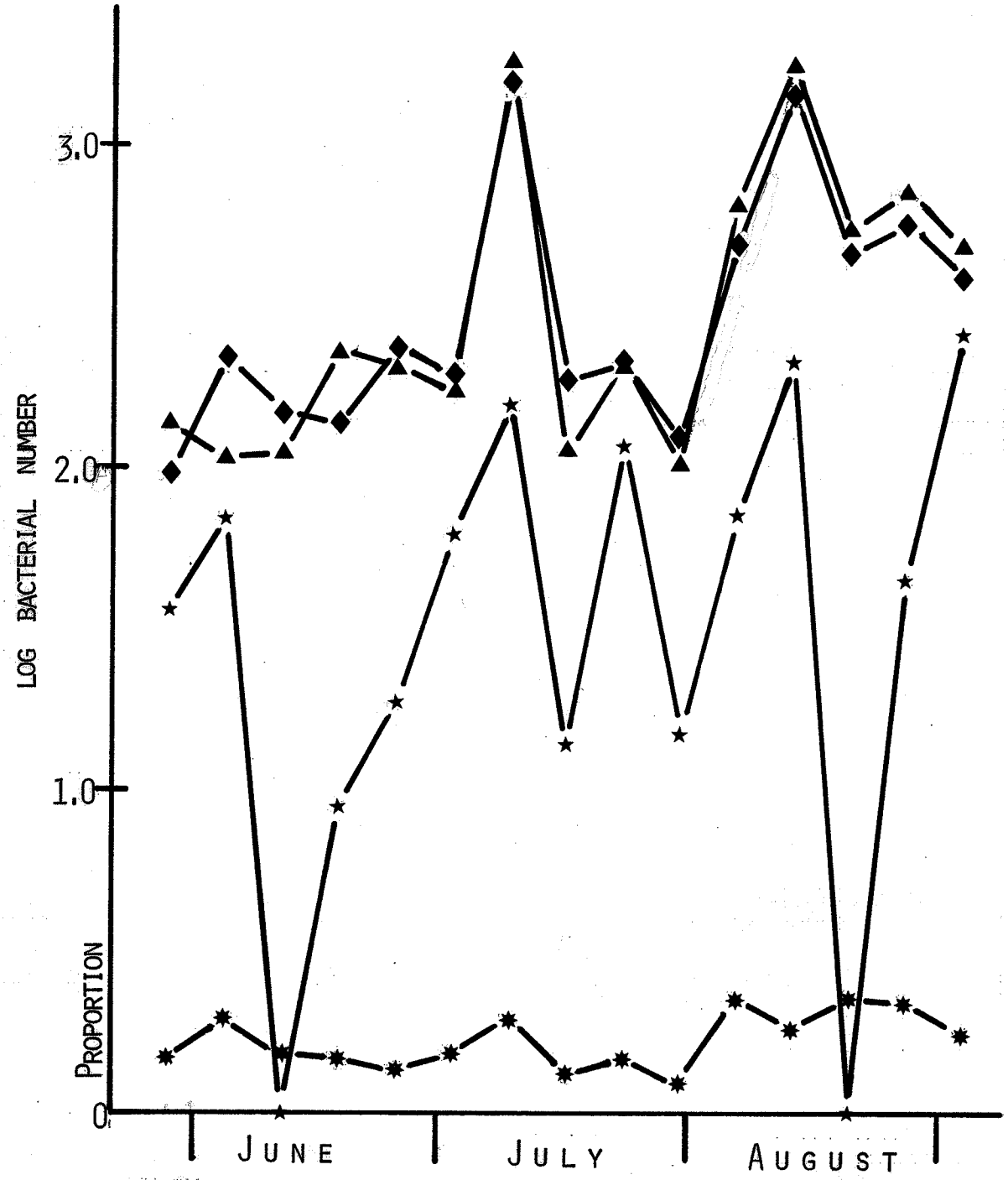

FIG.20: CHANGES IN THE MEAN VALUES OF THE UTILIZATION OF THE 9 ORGANIC ACIDS COMPARED WITH 2 CONSTITUENTS OF THE MEAN (1972).

◆—◆ MEAN OF 9 ORGANIC ACIDS

▲—▲ ACETATE

★—★ GLYCOLATE

✸—✸ MEAN PROPORTION FROM 0-1 OF 9 ACIDS

At times tartarate, citrate or pyruvate had counts low enough to significantly affect the correlation.

With respect to absolute counts and correlations succinate replica plating counts were more similar to glucose and glutamate then to the other organic acids. The bacterial preference for succinate is exceptional (Robinson etal 1973). Frequently from 0 - 17 meters the proportion of the Cytophaga agar counts able to utilize succinate as a substrate exceeded 0.5 particularly during August. The ability to utilize organic substrates varied as a proportion of the Cytophaga agar counts and was about 50% of Cytophaga agar counts during bacterial peaks.

DISCUSSION

Heterotrophy

The specificity of direct succinate counts and replica plating counts as opposed to a less selective medium such as Cytophaga agar permitted the obtaining of information vital in this study of the bacterial community.

A major disadvantage of replica plating counts as opposed to direct plate counts was that the potential counts were limited to those already on Cytophaga agar plates. The fact that the replica plated organisms grew on Cytophaga medium and that differences of numbers between different substrates were not very significant, nor were correlations much different between different substrates, indicated that these organisms were capable of growing on almost any medium. Bacterial types may have existed that were more specific with respect to substrate preferences, types that may not have grown on the nutrient rich Cytophaga medium. This flaw in replica plating could explain discrepancies between its preference order and the preference order obtained from turnover times

of ^{14}C uptake (Henzel M.S. 1972).

The phenomenon of replica plating counts being higher than direct plate counts of succinate is referred to as plating efficiency. This is defined as the differences in the ability of cells being able to form colonies on a complex as opposed to a simple medium, for instance, Cytophaga agar compared to succinate agar. Physiologically, the acclimatization of cells to Cytophaga agar facilitated their ability to utilize single organic substrates with respect to colonial numbers that were able to reform colonies on the single substrates after having been transferred. Since the natural environment is complex, comparable with Cytophaga medium, replica plating was probably a more accurate quantitative estimate of heterotrophic potential than direct inoculation. The possibility still existed that some bacterial types may have found Cytophaga medium too rich and preferred a simple substrate such as succinate when inoculated directly, to the exclusion of their being found on either Cytophaga medium or the replica plating plates.

Another source of doubt as to the accuracy and validity of the replica plating results was the fact that usually very few colonies were actually replicated, for the sake of clarity and ease of interpretation. Replica plating was dependent on one isolated plate, subjectively selected with usually less than the mean number of colonies for that dilution, while the direct inoculation used 3 plates with no real limit on the possible number of colonies on them.

Another line of evidence in favour of direct plate counts was the similarity of bacterial counts between epilimnial depths when directly inoculated succinate plate counts were used as opposed to the lack of comparable similarity with replica plating counts. Considering the physical nature of the epilimnion (turbulence and mixing) a correlation

of bacterial numbers between depths similar to that of chlorophyll a might have been expected.

Although Cytophaga medium may have selected for bacterial cells with a wide specificity range, differences between colonies and preference spectrums did exist. Usually the fluctuations of the replica plating counts for the various media were similar, but individual colonies were found to prefer one or more substrates and were unable to utilize others. The utilization patterns of the substrates seen each week were rarely specific for a single colonial type or for a single substrate, one exception being glutamate. For some colonies glutamate was the only assimilable and utilizable substrate. Usually the colonial types were able to utilize glutamate, glucose and a wide variety of organic acids. Other types utilized organic acids and either glutamate or glucose, but not both. Lactate utilization was unique to certain colonial types. Some of the colonies attained heavier growth on the single substrates than on the original Cytophaga agar plates.

Two major exceptions to the common seasonal pattern of replica plating numbers were lactate and glycolate. Lactate's utilization was zero for half of the season. The remainder of the season consisted of a number of minor peaks of which only the July 10th peak corresponded with the common pattern. Glycolate varied widely and particularly in June and August, although peaks did occur July 10th and August 14th in accordance with the other media. Glycolate and lactate appeared to be of minor importance as sources of energy for planktonic heterotrophic bacteria. A possible discrepancy might have been cells able to utilize these two acids, but unable to form colonies on Cytophaga medium.

The potential heterotrophy as assessed by replica plating and by direct inoculation onto succinate was probably related to the in situ

presence of the substrates tested. These substrates were probably released by an algal community fluctuating in 3 ways:

1. The algal biomass, for which chlorophyll a is at least a partial determinant, is varying causing changes in the ratio of particulates to soluble organics (bacterial substrates), assuming the particulates are breaking down.
2. The species composition of the lake is dynamic, and excretion is dependent on species. The carotenoid:chlorophyll a ratio may partially represent species changes, since each species may have a specific ratio.
3. The physiological state of the algal community changes, which is related to excretion and or senescence and autolysis, may also influence the carotenoid:chlorophyll a ratio and in turn may be represented by the ratio.

All 3 of these influence the concentration of the lake's soluble organic pool and consequently the numbers of bacteria supportable by the exosystem.

The counts of succinate utilizing organisms obtained from replica plating or direct inoculation can be examined as the sum of the 5 replica plating depths or each depth individually. The counts can be compared and related to chlorophyll a, the carotenoid:chlorophyll a ratio and temperature fluctuations. These contributing factors are linked to the presence of, and the rate of uptake of bacterial substrates.

The Cytophaga agar count relationships were quite similar to those seen with the means or sums of 0, 3, 7, 17, and 30 meters. Temperature changes were influential on chlorophyll a concentration changes and on the carotenoid:chlorophyll a ratio. Chlorophyll a decreases and pigment

ratio changes were linked to changes in bacterial numbers especially the July and August peaks. These 2 were common to all the 11 summed replica plating media.

Although basic relationships, especially chlorophyll a decreases accompanied by bacterial number increases, can be shown by summed replica plating data, only 2 examples were available. The sums tended to obscure individual examples and irregularities at each of the depths which may or may not have supported the relationships of the 4 parameters.

Examples existed in which succinate agar counts better fitted expected behaviour based on the observations of the Cytophaga agar count relationships than did the Cytophaga agar counts themselves. From 0 - 7 meters, during the early spring drop of chlorophyll a, Cytophaga agar counts dropped unexpectedly, while directly inoculated succinate agar counts rose. Bacterial substrates introduced at this time were most probably of a simple nature assimilable and metabolizable by the succinate utilizing populations. It may have been possible that the succinate counts of this time were not included in the Cytophaga agar counts, that is, 2 entirely different populations may have been enumerated by the 2 media at this time.

The results lead to the postulation that chlorophyll a decreases (excretion and/or senescence, autolysis and release of organic substrates) or high photosynthetic rates with relatively stable or increasing biomass (excess photosynthetic carbon appearing as excretory products) stimulate succinate number increases.

Temperature, particularly at the beginning and end of the sampling season, was negatively correlated to chlorophyll a. Many of the carote-

noid:chlorophyll a ratio changes appeared to be associated with temperature changes. Temperature may be a major controlling factor of not only algal biomass, but also species composition and physiological condition. Temperature indirectly controlled bacterial numbers by way of phytoplankton. There was no evidence of a simple direct relationship of temperature and bacterial numbers. Bacterial activity and temperature were positively correlated (Robinson etal 1973, unpublished) and this activity possesses the potential to initiate number increases by growth and reproduction. There appeared to be an association of the pigment ratio changes and bacterial number changes (succinate utilizing organisms), but the relationship was variable, being either positively or negatively correlated at any one time.

CONCLUSIONS

Methods

1. Plate counts were reliable particularly during bacterial peaks.
2. Cytophaga agar produced highest plate counts and directly inoculated succinate agar provided accessory information of a more specific nature, concerning the heterotrophic population.
3. The date provided circumstantial evidence for the validity of using chlorophyll a data, which may not measure accurately any isolated parameter such as biomass, but is intrinsically, nevertheless, ecologically significant.
4. Replica plating, although limited to Cytophaga agar colonies, showed that heterotrophic potential exists and is quantitatively correlated to the Cytophaga counts.

Cyclic patterns

1. At times a 14 day cycle did exist, but the weekly sampling interval was a drawback and may not always have detected such a cycle or a cycle of a similar or shorter duration. Discrete as opposed to continuous sampling may have indicated cycles that were artifacts.
2. Short cycles such as possible diurnal cycles would require continuous sampling and a refinement of the procedure from a statistical point of view (for example, more replicate plates).
3. A long term seasonal cycle did exist with spring and summer maxima of bacterial numbers.
4. The 4 epilimnial depths had very similar chlorophyll a patterns with a midsummer maximum following the spring drop.
5. The metalimnial and hypolimnial depths were all distinctive with

the exception of the early spring drop common to most of the depths.

6. On a mean basis 17 meters had a maximum chlorophyll a concentration dropping to a minimum at 30 meters.

Interrelations

1. Chlorophyll a and temperature were negatively correlated.

2. Apparently temperature was one of the major controlling factors of the phytoplankton community, including its times of maximum change coinciding to times of the carotenoid:chlorophyll a ratio changes.

3. Although bacterial peaks occurred for other reasons, many occurred in response to chlorophyll a decreases.

4. Temperature may have been a controlling factor in both species composition and physiological changes of phytoplankton.

Heterotrophy

1. As much as 50% and more of the Cytophaga organisms were capable of using each of 3 classes of substrates; an amino acid - glutamate, a sugar - glucose and an organic acid - succinate.

2. This proportion or percentage of the Cytophaga counts able to use the substrates was more constant for each substrate, than fluctuations of actual counts with time.

3. The colony per substrate order was as follows; glutamate (most colonies), glucose, succinate, malate, fumarate, pyruvate, acetate, citrate, tartarate, glycolate and lactate.

4. The results for glycolate and lactate indicated that these 2 were probably of minor significance in the nutrition of the heterotrophic bacterial community.

5. Direct inoculation of succinate and replica plating of succinate did

not correlate perfectly and may enumerate slightly different populations.

6. Replica plating produced consistently higher counts due to the phenomenon of plating efficiency.

7. The peaks of replica plating and direct inoculation counts coincided and probably bacterial increases were partially a result of increases of organisms potentially capable and probably currently existing on simple organic substrates in the lake.

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Appendix 1: Grouping of sampling dates for different sets of data and transformation to day of the year for purposes of computer analysis. For example, May 12, 1972 or 12/5 is transformed to day 133 of the 365 days of the year.

Day of Year	Sampling Dates		
	Bacteria	Pigments	Temperature
133	12/5	---	13/5
136	15/5	15/5	14/5
143	22/5	23/5	25/5
150	29/5	28/5	1/6
157	5/6	4/6	8/6
164	12/6	11/6	15/6
171	19/6	18/6	22/6
178	26/6	25/6	29/6
185	3/7	2/7	6/7
192	10/7	9/7	13/7
199	17/7	16/7	20/7
206	24/7	23/7	27/7
213	31/7	30/7	3/8
220	7/8	6/8	10/8
227	14/8	13/8	17/8
234	21/8	20/8	24/8
241	28/8	27/8	31/8
248	4/9	---	6/9
252	8/9	9/9	11/9

Appendix 2: Simple correlation coefficients between direct plate counts of a number of media for 1972 for days 133 to 252 inclusive, in West Blue Lake.

Depth	Cyt to TSA	Cyt to Suc	TSA to Suc
0 meters	0.936	0.716	0.873
1 meter	0.459	0.877	0.360
3 meters	0.667	0.246	0.860
5 meters	0.952	0.159	0.286
7 meters	0.881	0.794	0.977
12 meters	0.705	0.511	0.822
17 meters	0.790	0.677	0.872
25 meters	0.240	0.214	0.786
30 meters	0.438	0.110	0.489

Appendix 3: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 0 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	SLS	TSA	Succinate
12/5	1895		730	0
15/5	560		155	3
22/5	270	35	150	33
29/5	76	47	72	3
5/6	155	13	72	10
12/6	148	23	81	23
19/6	58	7	17	7
26/6	265	47	57	27
3/7	117	7	10	20
10/7	318	3	350	43
17/7	489	0	348	63
24/7	213	3	19	0
31/7	183	0	35	37
7/8	410	13	207	30
14/8	2242	10	1497	1033
21/8	138	0	108	33
28/8	385	17	310	100
4/9	245	17	177	40
8/9	413	20	293	203

Appendix 4: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 1 meter during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	830	0	560	
15/5	390	35	140	
22/5	180	10	90	15
29/5	87	0	133	37
5/6	121	17	65	20
12/6	102	3	53	15
19/6	115	10	38	47
26/6	424	20	32	93
3/7	73	3	42	7
10/7	1912	637	137	6
17/7	117	7	62	7
24/7	220	17	40	13
31/7	249	0	102	0
7/8	934	243	537	20
14/8	700	287	390	23
21/8	262	33	115	27
28/8	529	167	396	13
4/9	432	197	332	30
8/9	337	213	317	13

Appendix 5: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 3 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	5200	5	790	
15/5	950	10	360	
22/5	60	100	80	60
29/5	137	3	34	20
5/6	25	5	67	17
12/6	111	10	13	23
19/6	112	10	25	11
26/6	154	30	12	0
3/7	400	7	72	7
10/7	205	20	65	3
17/7	127	20	43	7
24/7	172	17	24	3
31/7	477	20	10	7
7/8	170	13	67	13
14/8	1747	667	975	50
21/8	242	23	72	17
28/8	263	83	198	23
4/9	289	60	177	23
8/9	1430	767	1320	20

Appendix 6: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 5 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	1610	3	650	
15/5	790	5	390	
22/5	130	30	160	35
29/5	88	0	48	17
5/6	46	13	45	13
12/6	172	7	135	77
19/6	72	13	32	37
26/6	165	33	38	203
3/7	163	17	102	10
10/7	290	73	114	17
17/7	80	3	42	7
24/7	225	3	14	13
31/7	112	20	37	10
7/8	210	27	117	10
14/8	393	93	157	56
21/8	315	107	115	13
28/8	307	33	108	27
4/9	757	197	432	17
8/9	343	173	237	13

Appendix 7: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 7 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	2060	3	550	
15/5	700	20	270	
22/5	280	30	100	50
29/5	150	0	32	47
5/6	58	0	58	13
12/6	194	23	37	10
19/6	132	30	85	50
26/6	342	20	95	367
3/7	135	7	43	13
10/7	2950	1500	3480	6
17/7	160	0	52	7
24/7	320	10	50	23
31/7	322	20	19	13
7/8	227	10	92	17
14/8	273	17	54	10
21/8	85	10	120	27
28/8	646	397	563	57
4/9	662	233	327	30
8/9	300	97	220	37

Appendix 8: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 12 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	580	20	210	
15/5	780	10	140	
22/5	210	0	80	70
29/5	80	0	55	27
5/6	15	3	30	10
12/6	195	10	45	30
19/6	8	3	0	0
26/6	387	13	54	50
3/7	230	3	50	47
10/7	157	27	79	20
17/7	198	3	33	7
24/7	207	90	142	27
31/7	100	3	52	17
7/8	753	177	208	7
14/8	165	30	156	30
21/8	27	13	41	10
28/8	502	170	290	13
4/9	323	157	225	0
8/9	60	7	40	3

Appendix 9: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 17 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	580	10	270	
15/5	280	5	100	
22/5	410	30	15	70
29/5	98	0	10	33
5/6	193	17	135	0
12/6	62	20	45	57
19/6	24	0	3	50
26/6	200	10	35	187
3/7	315	13	77	7
10/7	827	225	522	13
17/7	305	30	120	10
24/7	290	7	58	10
31/7	312	3	42	7
7/8	362	83	208	3
14/8	218	83	255	3
21/8	177	27	57	7
28/8	140	30	133	7
4/9	127	43	82	0
8/9	57	3	20	7

Appendix 10: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 25 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	280	33	160	
15/5	700	15	110	
22/5	130	5	35	25
29/5	80	0	35	13
5/6	120	13	118	7
12/6	187	10	50	10
19/6	20	3	28	51
26/6	172	40	53	33
3/7	469	73	252	33
10/7	303	60	154	6
17/7	158	23	95	17
24/7	269	13	95	20
31/7	307	3	10	3
7/8	494	27	42	43
14/8	433	33	143	20
21/8	339	57	60	27
28/8	325	7	102	43
4/9	219	120	292	10
8/9	150	17	157	7

Appendix 11: Bacterial numbers per milliliter of four media used for the direct inoculation of water column samples from 30 meters during 1972, West Blue Lake (12/5 equivalent to May 12, 1972).

Date	Cytophaga	Succinate	TSA	SLS
12/5	410	3	45	
15/5	200	250	130	
22/5	180	50	110	35
29/5	112	0	38	27
5/6	236	20	111	57
12/6	508	133	214	143
19/6	525	20	90	63
26/6	582	80	106	100
3/7	329	7	89	43
10/7	274	13	80	23
17/7	157	0	34	10
24/7	370	0	123	10
31/7	147	0	12	13
5/8	195	7	42	10
14/8	498	13	118	53
21/8	623	43	124	67
28/8	397	30	66	53
4/9	240	7	89	50
8/9	300	27	203	43

Appendix 12: Temperature date in degrees Centigrade for 1972, West Blue Lake, Manitoba (continued Table 13).

Date	0 meters	1 meter	3 meters	5 meters	7 meters
13/5	7.0	6.9	6.8	6.7	6.1
14/5	7.0	7.0	6.5	5.8	5.0
18/5	11.8	10.3	9.2	7.8	6.1
25/5	15.5	15.5	12.5	9.0	7.0
1/6	16.3	16.2	15.2	11.0	7.0
8/6	18.7	18.6	18.5	15.5	7.6
15/6	16.8	16.8	16.8	16.7	15.3
22/6	17.0	17.0	16.2	15.8	9.2
29/6	18.2	18.1	17.5	16.5	10.2
6/7	18.0	17.9	17.7	17.2	12.4
13/7	18.0	18.0	18.0	17.8	13.1
20/7	17.3	17.3	17.2	17.1	14.5
27/7	17.7	17.7	17.5	17.1	14.9
3/8	17.9	17.7	17.6	17.5	14.5
10/8	17.0	17.0	16.8	16.7	16.1
17/8	18.1	18.0	17.7	17.3	15.9
24/8	20.4	20.3	19.8	18.9	17.5
31/8	19.9	19.8	19.5	18.8	17.3
6/9	15.6	15.5	15.5	15.5	15.3
11/9	14.5	14.0	14.0	14.0	14.0

Appendix 13: Temperature data in degrees Centigrade for 1972.

Date	12 meters	17 meters	25 meters	30 meters
13/5	4.2		4.8	4.8
14/5	4.3	4.2	4.8	4.8
18/5	4.2		4.7	4.8
25/5	5.0		4.4	5.0
1/6	5.2		4.5	5.0
8/6	5.1	4.5	4.5	4.5
15/6	5.7	4.4	4.0	4.3
22/6	6.0		4.4	4.0
29/6			4.5	4.5
6/7			4.3	4.4
13/7			4.5	4.5
20/7			4.5	4.5
27/7		4.5		4.5
3/8			4.2	4.5
10/8			4.2	4.5
17/8	7.0		4.5	4.8
24/8	7.6		4.4	4.6
31/8	7.8		4.5	4.5
6/9	7.3	5.0	4.3	4.5
11/9	7.0		4.5	4.5

Appendix 14: Pigment data for 0 meters (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5	2.46	1.08	0.440
23/5	1.22	0.25	0.025
28/5	0.93	0.28	0.302
4/6	0.87	0.46	0.523
11/6	1.21	0.52	0.427
18/6	1.41	0.84	0.596
25/6	1.50	1.12	0.749
2/7	1.46	0.84	0.574
9/7	1.48	1.02	0.686
16/7	1.30	0.94	0.723
23/7	1.32	1.02	0.769
30/7	0.78	0.67	0.857
6/8	0.85	0.61	0.722
13/8	0.80	0.62	0.775
20/8	0.54	0.38	0.706
27/8	0.34	0.38	1.096
9/9	0.78	0.56	0.723

Appendix 15: Pigment data for 1 meter (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5	1.70	0.94	0.553
23/5	2.31	0.85	0.369
28/5	0.94	0.61	0.644
4/6	0.97	0.41	0.423
11/6	1.23	0.58	0.471
18/6	1.31	0.79	0.602
25/6	1.44	1.12	0.745
2/7	1.60	1.05	0.658
9/7	1.39	0.85	0.608
16/7	1.26	0.94	0.745
23/7	1.22	0.87	0.716
30/7	0.81	0.66	0.809
6/8	0.78	0.63	0.806
13/8	0.68	0.54	0.793
20/8	0.51	0.33	0.637
27/8	0.44	0.38	0.865
9/9	0.75	0.51	0.680

Appendix 16: Pigment data for 3 meters (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5	2.40	1.26	0.525
23/5	2.37	1.04	0.440
28/5	0.79	0.63	0.791
4/6	1.03	0.00	0.000
11/6	1.35	0.77	0.572
18/6	1.08	0.67	0.620
25/6	1.41	1.20	0.850
2/7	1.46	0.98	0.667
9/7	1.47	0.97	0.659
16/7	1.28	0.87	0.676
23/7	1.20	0.92	0.770
30/7	0.82	0.73	0.889
6/8	0.96	0.68	0.712
13/8	0.77	0.61	0.785
20/8	0.57	0.39	0.686
27/8	0.49	0.80	1.622
9/9	0.84	0.58	0.695

Appendix 17: Pigment data for 5 meters (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5	4.22	2.74	0.650
23/5	2.22	0.74	0.334
28/5	0.93	0.49	0.526
4/6	1.17	0.57	0.485
11/6	1.15	0.57	0.495
18/6	1.40	0.69	0.493
25/6	1.49	0.90	0.600
2/7	1.56	1.01	0.647
9/7	1.47	1.20	0.816
16/7	1.46	1.04	0.715
23/7	1.25	0.89	0.711
30/7	1.26	0.96	0.762
6/8	1.03	0.79	0.765
13/8	0.77	0.61	0.792
20/8	0.95	0.74	0.778
27/8	0.47	0.42	0.896
9/9	0.94	0.58	0.620

Appendix 18: Pigment data for 7 meters (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5	4.46	2.60	0.583
23/5	2.47	0.99	0.426
28/5	2.12	1.05	0.248
4/6	1.36	0.53	0.896
11/6	0.00	1.22	0.000
18/6	1.40	0.61	0.435
25/6	1.20	0.56	0.465
2/7	1.59	1.07	0.675
9/7	2.36	1.40	0.593
16/7	1.18	1.08	0.915
23/7	1.43	1.19	0.707
30/7	1.68	1.10	0.769
6/8	1.16	0.85	0.729
13/8	1.15	0.86	0.743
20/8	1.18	0.88	0.749
27/8	0.85	0.32	0.379
9/9	0.97	1.16	1.200

Appendix 19: Pigment data for 12 meters (1972) with concentrations in ug/1 for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5		3.90	0.540
23/5	3.37	1.68	0.498
28/5	1.82	1.43	0.783
4/6	2.34	1.26	0.539
11/6	1.39	0.52	0.375
18/6	1.93	0.73	0.375
25/6	1.55	0.76	0.486
2/7	1.88	1.09	0.581
9/7	1.72	0.90	0.521
16/7	1.40	0.65	0.462
23/7	1.32	0.78	0.588
30/7	1.05	0.68	0.648
6/8	1.19	0.70	0.586
13/8	0.96	0.66	0.687
20/8	0.96	0.56	0.586
27/8	0.85	0.56	0.656
9/9	1.14	0.68	0.596

Appendix 20: Pigment data for 17 meters (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5	2.80	1.46	0.523
23/5	2.33	0.84	0.361
28/5	0.95	0.16	0.168
4/6	1.03	0.10	0.092
11/6	1.16	0.00	0.000
18/6	1.30	0.46	0.355
25/6	1.04	0.44	0.418
2/7	1.08	0.76	0.703
9/7	1.55	0.74	0.476
16/7	1.92	0.84	0.435
23/7	2.49	1.24	0.497
30/7	3.00	1.67	0.555
6/8	2.26	1.19	0.525
13/8	0.97	0.60	0.619
20/8	0.87	0.37	0.428
27/8	0.88	0.46	0.520
9/9	1.08	0.56	0.514

Appendix 21: Pigment data for 25 meters (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5	2.22	1.02	0.460
23/5	2.08	0.53	0.254
28/5	0.55	0.13	0.228
4/6	0.52	0.00	0.000
11/6	0.49	0.00	0.000
18/6	0.57	0.19	0.334
25/6	0.43	0.25	0.570
2/7	0.40	0.73	1.840
9/7	0.63	0.26	0.416
16/7	0.78	0.33	0.426
23/7	0.82	0.37	0.445
30/7	1.22	0.66	0.536
6/8	1.34	0.75	0.556
13/8	1.44	0.78	0.543
20/8	1.56	0.84	0.536
27/8	0.42	0.15	0.362
9/9	0.33	0.15	0.440

Appendix 22: Pigment data for 30 meters (1972) with concentrations in ug/l for chlorophyll a and m-SPU for carotenoids, in West Blue Lake, Station 2.

Date	Chlorophyll a	Carotenoids	Carot:Chl a
15/5			
23/5	0.85	0.27	0.318
28/5	0.39	0.22	0.571
4/6	0.21	0.00	0.000
11/6	0.81	0.00	0.000
18/6	0.33	0.15	0.455
25/6	0.29	0.07	0.246
2/7	0.16	0.12	0.784
9/7	0.21	0.00	0.000
16/7	0.20	0.12	0.615
23/7	0.25	0.08	0.326
30/7	0.32	0.08	0.254
6/8	0.23	0.11	0.466
13/8	0.22	0.23	0.102
20/8	0.29	0.21	0.706
27/8	0.21	0.10	0.463
9/9	0.24	0.03	0.128

Appendix 23: Cytophaga agar and succinate agar direct plate counts in numbers per milliliter compared with three replica plating media (1972).[a] Sum of 0, 3, 7, 17, 30 meters, in West Blue Lake, Station 2.

	Direct Plating		Replica Plating		
Date	Cytophaga	Succinate	Succinate	Glucose	Glutamate
29/5	573	6	158	253	262
5/6	667	52	354	357	311
12/6	1023	209	319	332	614
19/6	851	67	309	591	339
26/6	1543	167	450	459	662
3/7	1296	41	373	389	487
10/7	4574	1801	2936	3232	3117
17/7	1238	113	471	386	474
24/7	1365	34	559	592	817
31/7	1441	80	460	258	679
7/8	1364	133	928	983	1247
14/8	4978	1813	3346	3229	3395
21/8	1265	137	589	390	924
28/8	1831	640	943	977	924
4/9	1563	383	726	994	746

a. The replica plate counts are determined by multiplying the proportion of the replicated Cytophaga plate able to grow on the specific substrate by the Cytophaga count per milliliter.

Appendix 24: The summed values for 0, 3, 7, 17, 30 meters of four replica plating media during 1972[a] at West Blue Lake, Station 2.

Date	Malate	Pyruvate	Acetate	Citrate
29/5	173	96	138	
5/6	358	282	107	326
12/6	289	239	112	88
19/6	434	231	230	83
26/6	420	464	205	347
3/7	318	317	174	107
10/7	2803	1651	1837	1663
17/7	325	196	113	100
24/7	520	432	209	88
31/7	95	258	104	62
7/8	851	802	657	301
14/8	2384	1745	1777	554
21/8	584	590	552	564
28/8	729	760	729	430
4/9	652		488	478

a. The replica plate counts are determined by multiplying the proportion of the replicated Cytophaga plate able to grow on the specific substrate by the Cytophaga count per milliliter.

Appendix 25: The summed values for 0, 3, 7, 17, 30 meters of four replica plating media during 1972[a] at West Blue Lake, Station 2.

Date	Fumarate	Tartarate	Glycolate	Lactate
29/5	60		36	
5/6	396	74	70	203
12/6	208	25	0	0
19/6	132	16	9	57
26/6	263	32	19	38
3/7	318	30	63	0
10/7	2336	428	158	98
17/7	346	32	14	0
24/7	322	51	118	8
31/7	126	94	15	0
7/8	636	164	71	13
14/8	1652	440	215	0
21/8	340	49	0	0
28/8	697	68	45	12
4/9	649	205	260	0

a. The replica plate counts are determined by multiplying the proportion of the replicated Cytophaga plate able to grow on the specific substrate by the Cytophaga count per milliliter.

Appendix 26: Simple correlation coefficients of Cytophaga agar counts and replica plating media counts (1972), (N = 14 from June 5th to September 4th) in West Blue Lake, Station 2.

	0 meters	3 meters	7 meters	17 meters	30 meters
Succinate	0.992	0.979	0.992	0.894	0.650
Glucose	0.993	0.937	0.986	0.881	0.425
Glutamate	0.990	0.944	0.990	0.942	0.811
Malate	0.974	0.956	0.988	0.889	0.773
Pyruvate	0.986	0.863	0.990	0.471	0.858
Acetate	0.977	0.928	0.992	0.870	0.669
Citrate	0.677	0.755	0.985	0.458	0.647
Fumarate	0.975	0.952	0.987	0.870	0.454

Appendix 27: Simple correlation coefficients of Succinate direct plate counts and replica plating media counts (1972), (N =14 from June 5th to September 4th) in West Blue Lake, Station 2.

	0 meters	3 meters	7 meters	17 meters	30 meters
Succinate	0.980	0.981	0.980	0.891	0.145
Glucose	0.985	0.968	0.970	0.886	0.104
Glutamate	0.981	0.959	0.974	0.885	0.596
Malate	0.967	0.983	0.975	0.878	0.316
Pyruvate	0.971	0.838	0.985	0.521	0.441
Acetate	0.978	0.953	0.988	0.953	0.469
Citrate	0.644	0.756	0.973	0.637	0.295
Fumarate	0.961	0.952	0.977	0.932	0.439

Appendix 28: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize glutamate from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	4/6	51	3/4	103	3/9	50	3/14	21	3/9	37
5/6	2/3	103	4/7	14	1/5	12	7/11	123	4/16	59
12/6	6/6	148	6/6	111	5/16[d]	54[d]	8/12	47	9/18	254
19/6	3/7	25	7/16	49	7/15	62	1/2	12	4/11	191
26/6	6/21	76	5/12	64	6/18	114	3/20	30	13/30	252
3/7	6/8	88	5/18	111	8/17	64	6/18	105	5/15	119
10/7	7/16	139	5/10	103	23/34	1995[c]	6/7	709[c]	15/24	171
17/7	16/38	206	4/8	64	3/8	60	8/17	144	0/15	0
24/7	7/12	124	11/22	86	10/13	246	12/13	268	7/28	93
31/7	6/12	92	5/15	159	4/13	99	19/31	197	18/20	132
7/8	30/35	352	9/9	410	8/16	114	20/28	258	11/19	113
14/8	14/21	1495[c]	7/9	1358[c]	10/13	210	12/14	187	7/24	145
21/8	6/12	69	10/13	186	5/9	147	7/10	124	4/5	498[c]
28/8	22/36	235	16/23	183	3/9	215[c]	10/11	127	12/29	164
4/9	4/9	109	7/15	135	4/7	378[c]	9/15	76	6/30	48

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 29: The proportions and the numbers of the replicated Cytophaga agarplate able to utilize glucose from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	5/6	63	3/4	103	1/9	17	1/14	70	0/9	0
5/6	2/3	103	4/7	14	2/5	23	9/11	158	4/16	59
12/6	6/6	148	5/6	93	2/16[d]	22[d]	3/12	13	2/18	56
19/6	3/7	25	6/16	42	4/15	35	1/2	12	1/11	48
26/6	5/21	63	3/12	39	6/18	114	1/20	10	12/30	233
3/7	4/8	59	4/18	89	6/17	48	6/18	105	4/15	88
10/7	9/16	179	6/10	123	26/34	2260[c]	5/7	591[c]	7/24	80
17/7	20/38	258	0/8	0	1/8	20	6/17	108	0/15	0
24/7	8/12	142	7/22	55	7/13	172	1/13	223	2/28	26
31/7	2/12	31	1/15	32	4/13	99	0/31	0	13/20	96
7/8	28/35	328	5/9	225	8/16	114	22/28	285	3/19	31
4/8	17/21	1820[c]	5/9	970[c]	9/13	189	8/14	125	6/24	125
21/8	7/12	81	9/14	156	5/9	47	6/10	106	0/5	0[c]
28/8	29/36	310	16/23	183	6/9	430[c]	8/11	102	11/29	151
4/9	5/9	136	5/15	96	7/7	662[c]	8/15	68	4/30	32

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 30: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize succinate from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	4/6	51	2/4	69	1/9	17	3/14	21	0/9	0
5/6	2/3	103	4/7	14	2/5	23	8/11	140	5/16	74
12/6	6/6	148	5/6	93	2/16[d]	22[d]	0/12	0	2/18	56
19/6	1/10	6	8/16	56	5/15	44	1/2	12	4/11	191
26/6	5/21	63	3/12	39	6/18	114	2/20	20	11/30	214
3/7	5/8	73	4/18	89	5/17	40	6/18	105	3/15	66
10/7	8/16	159	6/10	123	22/34	1910[c]	5/7	591[c]	14/25	153
17/7	21/41	250	5/8	79	2/8	40	6/18	102	0/15	0
24/7	7/12	124	8/22	63	5/13	123	1/13	223	2/28	26
31/7	4/12	61	5/15	222	3/13	74	0/31	0	14/20	103
7/8	29/35	340	8/9	151	8/16	114	17/28	220	10/19	103
14/8	15/21	1600[c]	6/9	1163[c]	8/13	168	8/14	125	14/24	290
21/8	6/12	69	9/13	167	5/9	47	5/9	98	2/6	208[c]
28/8	24/30	308	17/23	194	3/9	215[c]	7/11	89	10/29	137
4/9	5/9	136	5/15	96	4/7	378[c]	9/15	76	5/30	40

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 31: The proportions and the numbers of the replicated Cytophaga agar plateable to utilize malate from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	1/6	17	2/4	69	1/9	17	1/14	70	0/9	0
5/6	2/3	103	3/7	11	1/5	12	9/11	158	5/16	74
12/6	6/6	148	4/6	74	1/16[d]	11[d]	0/12	0	2/18	56
19/6	2/7	166	6/16	42	4/15	35	0/2	0	4/11	191
26/6	5/21	63	3/12	39	6/18	114	1/20	10	10/30	194
3/7	4/8	59	4/18	89	7/17	56	4/18	70	2/15	44
10/7	6/16	119	5/10	103	22/34	1910[c]	5/7	591[c]	7/24	80
17/7	14/38	180	1/8	16	0/8	0	6/17	108	2/15	21
24/7	7/12	124	6/22	47	5/13	123	1/13	223	1/28	13
31/7	2/12	31	1/15	32	1/13	25	0/31	0	1/20	7
7/8	25/35	293	8/9	151	5/16	71	18/28	233	10/19	103
14/8	10/21	1070[c]	5/9	970[c]	6/13	126	6/14	93	6/24	125
21/8	7/12	80	8/13	149	5/9	47	3/9	59	2/5	249[c]
28/8	23/36	246	11/23	126	2/9	144[c]	6/11	76	10/29	137
4/9	5/9	136	6/15	116	3/7	284[c]	9/15	76	5/30	40

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 32: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize fumarate from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	1/6	17	0/4	0	1/9	17	2/14	14	1/9	12
5/6	3/3	156	3/7	11	1/5	12	9/11	158	4/16	59
12/6	4/6	98	1/6	19	2/16[d]	22[d]	3/12	13	2/18	56
19/6	0/7	0	5/16	35	3/15	27	1/2	12	1/11	48
26/6	5/21	63	3/12	39	4/18	76	1/20	10	9/30	175
3/7	4/7	72	3/18	66	6/17	48	5/18	88	2/15	44
10/7	5/16	100	5/10	103	18/34	1560[c]	4/6	551[c]	2/24	22
17/7	16/36	217	1/8	16	1/7	23	5/17	90	0/15	0
24/7	7/12	125	8/22	63	4/13	99	1/13	22	1/28	13
31/7	2/12	30	3/15	96	0/13	0	0/31	0	0/20	0
7/8	18/35	210	7/9	132	4/16	56	16/28	207	3/19	31
14/8	10/21	1070[c]	3/9	582	6/13	126	5/14	78	4/24	83
21/8	4/12	46	9/13	167	5/9	47	4/9	80	0/5	0[c]
28/8	20/34	226	11/23	126	2/9	144[c]	5/11	64	10/29	137
4/9	5/9	275	4/15	77	2/7	189[c]	8/15	68	5/30	40

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 33: The proportions and the numbers of the replicated Cytophaga agar plateable to utilize pyruvate from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	3/6	38	1/4	34	1/9	17	1/14	7	0/9	0
5/6	1/3	52	3/7	11	0/5	0	1/11	175	3/16	44
12/6	3/6	74	5/6	93	1/16[d]	11[d]	1/12	5	2/18	56
19/6	2/7	17	5/16	35	4/15	35	0/2	0	3/11	144
26/6	5/21	63	2/12	26	5/18	95	1/20	10	9/30	270
3/7	4/7	61	3/18	67	5/17	40	6/18	105	2/15	44
10/7	7/16	139	5/10	103	14/34	1214[c]	1/6	138[c]	5/24	57
17/7	11/38	142	1/8	16	1/8	20	1/17	18	0/15	0
24/7	7/12	124	8/22	63	8/13	197	1/13	22	2/28	26
31/7	2/12	31	4/15	128	4/13	99	0/31	0	0/20	0
7/8	24/35	282	9/9	170	7/16	99	17/28	220	3/19	31
14/8	10/21	1070[c]	5/9	389[c]	3/13	63	6/14	98	6/24	125
21/8	6/12	69	10/13	186	5/9	47	2/9	39	2/5	249[c]
28/8	15/36	160	12/23	137	4/9	288[c]	3/11	38	10/29	137
4/9	6/9	163	4/15	77	2/7	189[c]	4/15	34	--	--

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 34: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize acetate from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	1/6	17	2/4	68	2/9	34	1/14	7	1/9	12
5/6	1/3	52	3/7	11	0/5	0	0/11	0	3/16	44
12/6	0/6	0	0/6	0	0/16[d]	0[d]	0/12	0	4/18	112
19/6	1/7	83	3/16	21	2/15	18	1/2	12	2/11	96
26/6	4/21	51	3/12	39	3/18	57	0/20	0	3/30	58
3/7	3/7	55	0/18	0	5/17	40	3/18	53	3/15	66
10/7	1/16	20	2/10	41	14/34	1214[c]	4/6	551[c]	1/24	11
17/7	7/37	93	0/8	0	1/8	20	0/17	0	0/15	0
24/7	5/12	89	1/22	8	4/13	99	0/13	0	1/28	13
31/7	1/12	15	2/15	64	1/13	25	0/31	0	0/20	0
7/8	19/35	222	6/9	113	5/16	71	17/28	220	3/19	31
14/8	10/21	1070[c]	3/9	582[c]	3/13	63	4/14	62	0/24	0
21/8	6/12	69	9/13	167	5/9	47	1/9	20	2/5	249[c]
28/8	17/35	187	11/23	126	3/9	215[c]	5/11	64	10/29	137
4/9	2/9	110	4/15	77	3/7	284[c]	2/15	17	0/30	0

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 35: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize citrate from June 5th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
5/6	2/3	104	3/7	11	1/5	12	8/11	140	4/16	59
12/6	2/6	49	0/6	0	1/16[d]	11[d]	0/12	0	1/18	28
19/6	0/7	0	2/16	14	1/15	9	1/2	12	1/11	48
26/6	3/21	38	2/12	26	4/18	76	1/20	10	5/30	97
3/7	1/7	17	1/18	22	3/17	24	0/18	0	2/15	44
10/7	5/16	100	5/10	103	15/34	1300[c]	1/6	138[c]	2/24	22
17/7	6/36	82	0/8	0	0/7	0	1/17	18	0/15	0
24/7	1/12	18	1/22	8	2/13	49	0/13	0	1/28	13
31/7	2/12	30	1/15	32	0/13	0	0/31	0	0/20	0
7/8	7/35	82	2/9	38	2/16	28	13/28	145	1/19	8
14/8	2/21	214[c]	1/9	194[c]	3/13	63	4/14	62	1/24	21
21/8	5/12	58	7/13	130	5/9	47	4/9	80	2/5	249[c]
28/8	15/34	170	6/23	69	1/9	72[c]	5/11	64	4/29	55
4/9	3/9	165	3/15	58	2/7	189[c]	4/15	34	4/30	32

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 36: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize tartarate from June 5th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
5/6	1/3	52	1/7	4	0/5	0	1/11	18	0/16	0
12/6	1/6	25	0/6	0	0/16[d]	0[d]	0/12	0	0/18	0
19/6	0/7	0	1/16	7	1/15	9	0/2	0	0/11	0
26/6	1/21	13	0/12	0	1/18	19	0/20	0	0/30	0
3/7	0/7	0	1/18	22	1/17	8	0/18	0	0/15	0
10/7	2/16	40	2/10	41	4/34	347[c]	0/6	0[c]	0/24	0
17/7	1/35	14	0/7	0	0/7	0	1/17	18	0/15	0
24/7	1/2	18	1/22	8	1/13	25	0/13	0	0/28	0
31/7	2/12	30	2/15	64	0/13	0	0/31	0	0/20	0
7/8	3/35	35	2/9	38	3/16	42	3/28	39	1/19	10
14/8	0/21	0[c]	2/9	388[c]	1/13	21	2/14	31	0/24	0
21/8	0/12	0	0/13	0	1/9	9	2/9	40	0/5	0[c]
28/8	4/34	45	2/23	23	0/9	0[c]	0/11	0	0/29	0
4/9	2/9	110	0/15	0	1/7	95[c]	0/15	0	0/30	0

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 37: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize glycolate from May 29th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
29/5	0/6	0	0/4	0	1/9	17	1/14	7	1/9	12
5/6	1/3	52	0/7	0	0/5	0	1/11	18	0/16	0
12/6	0/6	0	0/6	0	0/16[d]	0[d]	0/12	0	0/18	0
19/6	0/7	0	0/16	0	1/15	9	0/2	0	0/11	0
26/6	0/21	0	0/12	0	1/18	19	0/20	0	0/30	0
3/7	1/7	17	1/18	22	3/17	24	0/18	0	0/15	0
10/7	2/16	40	0/10	0	2/34	174[c]	0/6	0[c]	4/24	44
17/7	1/35	14	0/7	0	0/7	0	0/17	0	0/15	0
24/7	3/12	54	2/22	16	2/13	48	0/13	0	0/28	0
31/7	1/12	15	0/15	0	0/13	0	0/31	0	0/20	0
7/8	3/35	35	0/9	0	0/16	0	2/28	26	1/19	10
14/8	0/21	0[c]	1/9	194[c]	1/13	21	0/14	0	0/24	0
21/8	0/12	0	0/13	0	0/9	0	0/9	0	0/5	0[c]
28/8	4/34	45	0/23	0	0/9	0[c]	0/11	0	0/29	0
4/9	3/9	165	0/15	0	1/7	95[c]	0/15	0	0/30	0

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.

Appendix 38: The proportions and the numbers of the replicated Cytophaga agar plate able to utilize lactate from June 5th to September 4th of 1972, in West Blue Lake, Station 2.

Date	0 meters		3 meters		7 meters		17 meters		30 meters	
	Prop.[a]	No.[b]	Prop.	No.	Prop.	No.	Prop.	No.	Prop.	No.
5/6	2/3	104	1/7	4	0/5	0	2/11	36	4/16	59
12/6	0/6	0	0/6	0	0/16[d]	0[d]	0/12	0	0/18	0
19/6	0/7	0	0/16	0	1/15	9	0/2	0	1/11	48
26/6	0/21	0	0/21	0	1/18	19	0/20	0	1/30	19
3/7	0/7	0	0/18	0	0/17	0	0/18	0	0/15	0
10/7	0/16	0	0/10	0	1/34	87[c]	0/6	0[c]	1/24	11
17/7	0/34	0	0/7	0	0/7	0	0/17	0	0/15	0
24/7	0/12	0	1/22	8	0/13	0	0/13	0	0/28	0
31/7	0/12	0	0/15	0	0/13	0	0/31	0	0/20	0
7/8	0/35	0	0/9	0	0/16	0	1/28	13	0/19	0
14/8	0/21	0[c]	0/9	0[c]	0/13	0	0/14	0	0/24	0
21/8	0/12	0	0/13	0	0/9	0	0/9	0	0/5	0[c]
28/8	0/34	0	1/23	12	0/9	0[c]	0/11	0	0/29	0
4/9	0/9	0	0/15	0	0/7	0[c]	0/15	0	0/30	0

(a) The denominator is the number of colonies on the original Cytophaga agar plate replicated successfully onto the control Cytophaga agar plate.

(b) The number per milliliter is calculated by multiplying the proportion obtained from a single plate, by the mean number of Cytophaga agar colonies for that particular depth and time.

(c) The Cytophaga agar plate replicated was chosen from the 10^1 dilution.

(d) A 5 meter Cytophaga agar plate was substituted.